Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

REPLY MOTION RECORD OF THE DEFENDANT, DAVID FISMAN - Vol. 1

(Returnable November 19, 2024)

March 15, 2023

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Plaintiff

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UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

REPLY AFFIDAVIT OF DR. DAVID FISMAN

I, Dr. David Fisman, of the City of Toronto, in the Province of Ontario, MAKE OATH AND SAY:

1. I am one of the Defendants in this proceeding, and, as such, have knowledge of the matters contained in this Affidavit. Where my knowledge is based on information and belief, I state the source of that information and verily believe it to be true.

2. I have had the opportunity to review the affidavit of Dr. Byram Bridle sworn December 15, 2023 (the "Bridle Affidavit"), the affidavit of Dr. Bonnie Mallard, sworn December 15, 2023, the affidavit of Dr. David Speicher sworn December 13, 2023, the affidavit of Dr. Harvey Risch affirmed December 11, 2023, , the affidavit of Dr. Steve Pelech, sworn December 1, 2023, the affidavit of Dr. Pierre Major, sworn November 29, 2023 and the affidavit of Niel Karrow affirmed December 15, 2023 (collectively the "Responding Affidavits").

3. I swear this affidavit in reply to the Responding Affidavits.

Purpose of my Twitter Account in 2021

4. As explained in paragraph 3 of my Affidavit sworn May 26, 2023, in May and June 2021, I was a member of Ontario's COVID-19 Science Advisory Table (the "Science Table").

5. As set out on its website, the purpose of the Science Table was to "evaluate and report on emerging evidence relevant to the COVID-19 pandemic, to inform Ontario's response", and to provide "credible and independent scientific and technical advice to inform government and the broader public". A copy of the "About Us" page of the Science Table is attached hereto as **Exhibit "A"**.

6. Because of my public facing role as a member of the Science Table, I gained a large Twitter following during the pandemic. I frequently tweeted my opinion on scientific issues that arose during the pandemic. My intention in tweeting this was to provide the public with information that I felt was truthful and accurate during an unprecedented global pandemic. At all time, my intention was to ensure my Twitter followers had, what I believed to be, accurate information on the risks of COVID-19, and information on how best to protect themselves.

7. In May 2021, I had a genuine and honest belief that the science relied on by Dr. Bridle in his radio interview with Alex Pierson on AM640 was not data based and was misinformation. My intention in advising my Twitter followers of my opinion on Dr. Bridle's claims was to encourage the public to obtain their COVID-19 vaccination, which I thought, and still believe, is in the public interest.

8. Attached as **Exhibit "B"** is a copy of the transcript of Dr. Bridle's AM640 radio interview with Alex Pierson from May 27, 2021.

9. I had no intention of causing Dr. Bridle harm through my tweets. At no time did I accuse Dr. Bridle of being dishonest.

Response to the Medical Claims in the Responding Affidavits

10. Within the Bridle Affidavit, Dr. Bridle makes a number of claims about the COVID-19 vaccine, including:

(a) Cases of COVID-19 were disproportionately diagnosed among people that had received COVID-19 vaccines, and that hospitalizations occurring due to COVID-19 were disproportionately higher among those that received COVID-19 vaccines;[1]

(b) The spike protein circulating in blood might be able to cause harm; [2] and

(c) There is substantial evidence about the role of the vaccine spike protein circulating to and causing pathogenic reaction in various organs.[3]

11. It is unfortunate that Dr. Bridle, even in this litigation, continues to misconstrue the medical evidence on the efficacy of vaccines.

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[1] Responding Motion Record, page 97
[2] RMR, page 106, Bridle Affidavit, para 26
[3] RMR, page 105, Bridle Affidavit, page 27

12. As recently as February 24, 2024, Dr. Bridle also published an article on his COVID Chronicles blog titled "Health Canada Hid Their Concerns About Impurities in COVID-19 Shots from Canadians", a copy of which is attached hereto as **Exhibit "C"**.

13. Contrary to Dr. Bridle's comments in the Bridle Affidavit, the COVID-19 vaccine has been deemed safe and effective at preventing serious complications associated with contracting COVID-19. For example:

(a) Attached hereto as **Exhibit "D"** is an article from Scientific America titled "Vaccination Dramatically Lowers Long COVID Risk". As set out in the article, a study published in November in the BMJ, a weekly medical journal, found that a single COVID vaccine dose reduced the risk of long COVID by 21 percent, two doses reduced it by 59 percent and three or more doses reduced it by 73 percent;

(b) Attached hereto as **Exhibit "E"** is an article titled "Real-World Effectiveness of BNT162b2 Against Infection and Severe Diseases in Children and Adolescents" from Annals of Internal Medicine which shows that the risk of cardiac events is more likely in unvaccinated children than in vaccinated children;

(c) Attached hereto as **Exhibit "F"** is an article which I co-authored, titled "Severity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in Pregnancy in Ontario: A Matched Cohort Analysis", from the Clinical Infectious Diseases journal dated February 1, 2023. The article states that given the markedly elevated risk of hospitalization and intensive care admission for pregnant women

infected with COVID-19, and the safety of SARS-CoV-2 vaccines in pregnancy, a risk-benefit calculus strongly favours vaccination in pregnant women;

(d) Attached hereto as **Exhibit "G"** is an article which I co-authored titled "Relative Virulence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Among Vaccinated and Unvaccinated Individuals Hospitalized With SARS-CoV-2" from the Clinical Infectious Diseases journal from February 1, 2023. As set out in the article, even when vaccines failed to prevent infection severe enough to require hospitalization, the virulence of COVID-19 infections in those individuals was decreased;

(e) Attached hereto as **Exhibit "H"** is an article which I co-authored titled "Vaccine effectiveness against hospitalization among adolescent and pediatric SARS-CoV-2 cases between May 2021 and January 2022 in Ontario, Canada: A retrospective cohort study" published on March 31, 2023 in Plos One, an open access, peer reviewed journal. As set out in the article, two mRNA vaccine doses in adolescents were associated with an 85% lower likelihood of hospitalization among SARS-CoV-2 cases caused by the Omicron variant. Among children, one mRNA dose was associated with a 79% lower likelihood of hospitalization among SARS-CoV-2 cases caused by the Omicron variant; and

(f) Attached hereto as **Exhibit "I"**, is a 2023 article titled "The role of COVID-19 vaccines in preventing post-COVID-19 thromboembolic and cardiovascular complications" which shows that the COVID-19 vaccinations provide strong protetion against heart attacks, strokes, venous thromboembolism and heart failure.

14. Contrary to Dr. Bridle's assertions, I am a physician and considered an expert in COVID-19 related topics, including vaccines. Recently, on January 22, 2024, my medical research was cited by JP Morgan Asset Management in an article that was sent to their clients on vaccines and COVID-19 related topics. A copy of their article titled "Eye on the Market" is attached hereto as **Exhibit "J"**.

My Tweets and Actions on Twitter

15. During the pandemic, there were a number of clinicians, professors and scientists who were active on Twitter and were trying to promote data-based evidence on issues like mask mandates and vaccines.

16. While I became involved in that community, and came to know many of those scientists who were promoting data-backed scientific information, I deny that I was ever involved in a conspiracy to harm Dr. Bridle. Instead, I continued to independently tweet my opinions on Twitter, which sometimes included referencing colleagues who were tweeting information on the COVID-19 pandemic.

17. On May 10, 2021, I retweeted an article from readpassage.com titled "Media Has Ignored the Anti-Vax Movement's White Supremacist Roots". Along with the article, I wrote:

> A good read from @Notone on close linkages between antivaxxers, antimaskers and neo-Nazis in Canada. Media needs to start calling this for what it is. H/t @h_shriya

18. I did not write the underlying article nor did I mention Dr. Bridle in my tweet. I also had no involvement in what other independent Twitter users commented on my tweet.

19. Contrary to the allegation in the Bridle allegation, I had no involvement in the creation of the website byrambridle.com. I recall that, on May 29, 2021, I received a direct message from a Twitter user who I did not know, which directed me to the byrambridle.com website. I recall that this was the first time I learned about the website. After reviewing the website, I saw that it contained data-based research which countered the comments Dr. Bridle had been publicly making. I felt it contained accurate and valuable information and accordingly retweeted it to my followers.

20. On August 19, 2021, I tweeted:

> @uofg administration has been absolutely bizarre for months now.
>
> They've coddled spreaders of vaccine misinformation on campus, even as they've created safety issues (not just related to vaccination) for others in the @uofg university community.

21. Contrary to Dr. Bridle's suggestion, I did not tweet this comment to support Dr. Bridle's "removal from campus" as alleged in paragraph 109 of the Bridle Affidavit, but rather after the University of Guelph had declined to respond to my concerns that Dr. Bridle was encouraging his supporters to harass me, and after I learned that a University of Guelph professor, Amy Greer, had been assigned security in light of her pro-vaccine views.

22. Any loss of income associated with Dr. Bridle's employment with the University of Guelph appears not to be related to anything I have said, but instead is a direct result of Dr. Bridle refusing to be vaccinated.

23. On September 17, 2021, Dr. Bridle wrote an open letter to the President of the University of Guelph, a copy of which is attached hereto as **Exhibit "K"**. As set out in the open letter, Dr.

Bridle acknowledges that he had been banned from the University of Guelph's campus because he failed to adhere to the University's vaccine mandate, stating: "you have banned me from the campus and ruined my life because I don't have a piece of paper saying that someone saw two needles go into my shoulder".

24. A copy of the University of Guelph's COVID-19 Vaccination Policy is attached hereto as **Exhibit "L"**. As set out in the policy, it first came into effect of September 7, 2021, and was paused on May 1, 2022.

25. Dr. Bridle asserts that as a result of my tweets he has been unable to obtain grant funding. I understand that the reason Dr. Bridle has been unable to conduct research and obtain grant funding is because he has not been permitted to attend at his laboratory at the University of Guelph. I was in no way involved in this decision nor have the power or ability to control his access to the laboratory, funding or graduate students.

26. Further, I am a former chair of a Canadian Institutes of Health Research institute. In that role, I learned that the success rate of scientists requesting to receiving grants is approximately 17%.

Dr. Bridle's Reputation is of his own Making

27. Independent of my tweets, Dr. Bridle's opinions on COVID-19 vaccines have garnered significant criticism.

28. On June 7, 2021, David Gorski wrote an article for the website "Science-Based Medicine" titled "COVID-19 vaccines are going to sterilize our womenfolk, Take 2", in which Dr. Bridle was described as an "antivaxxer, antimasker, and COVID-19 conspiracy theorists and has made a

number of false claims about vaccines dating back to the early days of the pandemic." A copy of Mr. Gorski's article dated June 7, 2021 is attached hereto as **Exhibit "M"**.

29. On December 7, 2022, Global News published an article by Kathryn Mannie titled "Court intervenes after baby's parents refuse 'vaccinated blood' transfusion". As set out in the article, Dr. Bridle had sworn an affidavit in support of parents in New Zealand who would not consent to life-saving surgery for their six-month-old child, over fears that the baby would receive "vaccinated blood", and were seeking a court order that baby only receive blood from unvaccinated donors. During the trial, Dr. Bridle's credentials were criticized, and Ms. Mannie's article described Dr. Bridle as a "controversial Canadian academic" who "has faced criticism from the scientific and medical community". A copy of Ms. Mannie's article dated December 7, 2022 is attached hereto as **Exhibit "N"**.

30. On January 22, 2024, David Gorski wrote an article for the website "Science-Based Medicine" titled "New school" antivax goes old school as Byram Bridle asks if COVID-19 vaccines will drive an "epidemic" of autism". A copy of Mr. Gorski's article dated January 22, 2024 is attached hereto as **Exhibit "O"**.

31. On August 4, 2021, Dr. Bridle appeared on Laura Ingraham's Fox News show. In response, an Eric Kleefeld wrote an article for Media Matters for America describing Dr. Bridle as a "fearmonger" whose arguments have been described as misleading by experts, as "he is cherry-picking data from studies that show the vaccines actually working properly." A copy of Mr. Kleefeld's article title "Laura Ingraham guest pushes debunked claims that the COVID-19 vaccines are a "toxin"" is attached hereto as **Exhibit "P"**.

32. In any event, it is not clear that Dr. Bridle has suffered any harm to his reputation. In Dr. Bridle's biography on the website for an event called "Shifting Perspectives", Br. Bridle claims to have participated in "~250 media engagements ranging from radio shows, published articles, and appearances on televised news programs (including, but not limited to, W5, The West Block, CTV National News, and Fox News), spanning the local to international scope", and that "[d]uring the declared pandemic, Dr. Bridle has published thirty-three peer-reviewed papers in high-quality indexed scientific journals, including three that are solely focused on COVID-19."

33. A copy of Dr. Bridle's biography from the Shifting Perspectives website is attached hereto as **Exhibit "Q"**.

34. Shifting Perspectives was an event that occurred on April 30, 2022 which featured both Dr. Bridle and Dr. Mallard.

35. These figures were updated in Dr. Bridle's biography on the website for the "What Would Christine Anderson Do? Canadian Tour" event. Dr. Bridle appeared with Ms. Anderson at her February 21, 2023 event in Toronto. A copy of Dr. Bridle's biography from that website, which states he was had ~300 media engagements since the COVID-19 pandemic was declared, is attached hereto as **Exhibit "R"**.

Dr. Bridle has Not Suffered any Loss

36. Based on publicly available documents, Dr. Bridle has continued to receive income and grants, despite his position on this motion.

37. Attached hereto as **Exhibit "S"** is a copy of Dr. Bridle's information on Ontario's 2023 Sunshine List. As set out in the Sunshine List, Dr. Bridle continued to receive income from the

University of Guelph in 2021-2023. Instead of a decrease in annual salary, each year Dr. Bridle's income from the University of Guelph appears to have increased from $136,881.92 in 2021 to $138,695.78 in 2023.

38. In addition, Dr. Bridle has continued to receive grants since 2021. The Government of Canada's Awards Database, a publicly available website, lists Dr. Bridle as having received a $32,000 award from the Natural Sciences and Engineering Researching Council of Canada (NSERC) in 2021 for his research project titled "Calming the Cytokine Storm: Elucidating Mechanisms Contributing to Toxic Inflammatory Responses to Viruses". A copy of NSERC's Awards Database page showing this grant is attached hereto as **Exhibit "T".**

39. Dr. Bridle also received a research grant from the Cancer Research Society in or around September 2021. A copy of the Cancer Research Society's website announcing Dr. Bridle as a recipient is attached hereto as **Exhibit "U"**.

Dr. Bridle's "experts" are not impartial

40. Contained within the Responding Material, Dr. Bridle has served the affidavit of Dr. Bonnie Mallard, sworn December 15, 2023, the affidavit of Dr. David Speicher sworn December 13, 2023, the affidavit of Dr. Harvey Ricsh affirmed December 11, 2023, the affidavit of Dr. Steve Pelech, sworn December 1, 2023, the affidavit of Dr. Pierre Major, sworn November 29, 2023 and the affidavit of Niel Karrow affirmed December 15, 2023.

41. I am aware of these physicians, as throughout the pandemic, they were all vocal opponents of mask mandates, COVID-19 vaccines and government shut-downs.[4]

42. The Affidavits Dr. Bridle has tendered are not impartial experts capable of giving unbiased evidence to the court. This group of experts has previously worked together and with Dr. Bridle:

(a) Attached hereto as **Exhibit "V"** is a copy of the 2021 article "Maternal COVID-19 Vaccination and its Potential Impact on Fetal and Neonatal Development", published in the journal "Vaccines", an open access journal published monthly online by MDPI, authored by Dr. Karrow, Dr. Pelech, Dr. Bridle and Dr. Mallard among others;

(b) Attached hereto as **Exhibit "W"** is a copy of the 2022 article "Immunoceuticals: Harnessing Their Immunomodulatory Potential to Promote Health and Wellness", published in the journal "Nutrients", an open access journal published monthly online by MDPI, authored by Dr. Bridle, Dr. Karrow and Dr. Mallard, among others;

(c) Attached hereto as **Exhibit "X"** is a copy of the 2023 article "N-Acetylcysteine and its Immunomodulatory Properties in Humans and Domesticated Animals",

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[4] Ugolini, Tamara, "'Health Canada hasn't considered the risk' of COVID vaccine DNA contamination issue, says citizen scientist" October 25, 2023 < https://www.rebelnews.com/health_canada_hasnt_considered_the_risk_of_covid_vaccine_dna_contaminants>; Zimmer, Charlotte, "YSPH professor criticized for promoting unproven drug to treat COVID-19" Janaury 22, 2024, Yale News; Shuttleworth, Joanne, "U of G scientists concerned about extended internal between COVID-19 vaccine doses" 24 March 2021, The Wellington Advertiser.

published in the journal "Antioxidants", an open access journal published monthly online by MDPI authored by Dr. Bridle, Dr. Karrow and Dr. Mallard, among others;

(d) Dr. Bridle and Dr. Major have published six articles together between 2017-2022, including the article "Intranasal vaccination with a Newcastle disease virus-vectored vaccine protects hamsters from SARS-CoV-2 infection and disease", a copy of which is attached hereto as **Exhibit "Y"**;

(e) Dr. Bridle and Dr. Major have also worked together on a US patented Newcastle Disease Virus vector. A copy of Dr. Major's patent listings which includes this patent with Dr. Bridle is attached hereto as **Exhibit "Z"**;

(f) Drs. Bridle, Karrow, Speicher and Pelech, among others, published an article titled "Concerns regarding the efficacy and safety for BNT162b2 mRNA coronavirus disease (COVID-19) vaccine through six months" in 2022 with the Canadian Covid Care Alliance, a copy of which is attached hereto as **Exhibit "AA"**;

(g) Dr. Bridle, Karrow and Mallard worked together to create a nine-lecture course webinar called "Introduction to Immunology and Infectious Disease" with a Guelph-based organization called ImmunoCeutica Inc. A copy of the digital advertisement for the ImmunoCeutica course is attached hereto as **Exhibit "BB"**; and

(h) Attached hereto as **Exhibit "CC"** is an article posted on The Federalist website titled "Forcing People Into COVID Vaccines Ignores Important Scientific Data" dated December 14, 2021, written by Dr. Risch and Dr. Bridle, among others.

43. Finally, I note that even after the commencement of this litigation and the delivery of my materials on this motion, Dr. Bridle has continued to make disparaging comments about my work on his COVID Chronicles Substack blog. On December 14, 2023, Dr. Bridle published an article titled "Guess What? Segregation of Unvaccinated People to Protect the "Vaccinated" is NOT Supported by Modeling Practiced With Integrity", a copy of which is attached hereto as **Exhibit "DD"**. In that article, Dr. Bridle referred to my April 25, 2022 article titled "Impact of population mixing between vaccinated and unvaccinated subpopulations on infectious disease dynamics: implications for SARS-CoV-2 transmission" published in the Canadian Medical Association Journal in such terms:

> April 25, 2022, is a day that will live on in infamy within the sphere of Canadian science. It is the day that the worst piece of trash that I have ever seen was published in the Canadian Medical Association Journal. This paper, which can serve no more useful purpose than wiping one's soiled butt crack, can be found here.

44. I swear this affidavit in support of my motion to dismiss the Claim under s. 137.1 of the *Courts of Justice Act* and for no improper purpose.

SWORN by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024 in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits
(or as may be)

KATHERINE R. COSTIN

DAVID FISMAN

This is Exhibit A referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15 , 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

SCIENCE TABLE
COVID-19 ADVISORY FOR ONTARIO

[https://covid19-sciencetable.ca]

Menu

Science Briefs [https://covid19-sciencetable.ca/science-briefs/]Ontario Dashboard [https://covid19-sciencetable.ca/ontario-dashboard/]Key Resources [https://covid19-sciencetable.ca/key-resources/]Glossary [https://covid19-sciencetable.ca/glossary/]About Us [https://covid19-sciencetable.ca/about/]Our Partners [https://covid19-sciencetable.ca/our-partners/]

NOTICE: This website is no longer updated and the resources on this site are outdated.
If you have questions about previously published Ontario COVID-19 Science Advisory Table resources, please email communications@oahpp.ca.

A Collective Lens.

Informing Ontario's response to COVID-19

About the Science Table

The Ontario COVID-19 Science Advisory Table is a group of scientific experts and health system leaders who evaluate and report on emerging evidence relevant to the COVID-19 pandemic, to inform Ontario's response.

The Science Advisory Table's mandate is to provide summaries of relevant scientific evidence to public health and health care professionals, as well as the general public, by integrating information from existing scientific tables, Ontario's universities and agencies, and the best global evidence.

The Science Advisory Table is hosted by Public Health Ontario (PHO). Aligned with PHO's mandate, the Science Advisory Table provides credible and independent scientific and technical advice to inform government and the broader public as Ontario transitions to recovery and to help prepare for and respond to future public health emergencies.

Terms of Reference of the Ontario COVID-19 Science Advisory Table [https://covid19-sciencetable.ca/wp-content/uploads/2022/03/Terms-of-Reference-for-the-Science-Table.pdf]

Science Table Members

Leadership

This is Exhibit B referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 1 5 , 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Transcription of Podcast:

https://podcasts.apple.com/ca/podcast/new-peer-reviewed-study-on-covid-19-vaccines-suggests/id1318830191?i=1000523346577

The Alex Pierson Show: New peer reviewed study on COVID-19 vaccines suggests why heart inflammation, blood clots and other dangerous side effects occur

Speaker	**Context**
Alex Pierson	I'm talking about a lot of science these days. It's coming at us fast and furious, and a lot of people asking a lot of good questions. You know, the vaccines, are they safe for kids? Certainly there's a big push to get kids as young as 12 the shot as soon as possible but not everyone's confident about it, even if you're not an anti vaxxer. There are a lot of parents who are kind of nervous about putting something into their kids and then I read that there have been several dozen cases of heart problems in teens and young adults, which Israel is now looking into, and what they're looking into, which they'll release the results of are why mostly males, not all, but around 22 years of age and younger, are getting heart inflammation. So one to four days after getting the shot, they get like a shortness of breath and fatigue and some very specific chest pain. It's mild, so no one's gotten really sick or died but you wanna know, what you don't know, if you're going to put something into your kids. Let's bring in Doctor Byron Bridal, he's an associate professor of viral immunology at the University of Guelph. Doctor, you've been very, you know, very open on this whole issue and and you know you're not an anti vaxxer by any stretch but what do you think about this inflammation in the heart and, and is it an actual threat?
Dr. Byram Bridle-	Thanks for having me, Alex, uh, yeah, as you said, I'm I'm very much pro vaccine but always making sure that the science is done properly and that we follow the science carefully before going into, you know, public rollout of vaccines. I hope you run, let me run with this a little bit, Alex. I'll provide, I, I can, I'll forewarn you and your, your listeners that the story I'm about to tell is a bit of a scary one. This is cutting edge science. There's a couple of key pieces of scientific information that have become privy to just within the past few days that has made the final link so we understand now. Myself and some key international collaborators, we understand exactly why these problems are happening and many others associated with these vaccines and the story is a bit of a scary one so just embrace you for this, but I'm going to walk you through this. The the science that I'm going

Speaker	**Context**
	to be talking about, I don't have the time here to describe exactly the scientific data, but let me assure you that everything that I'm stating here, that I'm stating right now, is completely backed up by peer reviewed scientific publications in well known and well respected scientific journals. I have all of this information in hand. I'm in the process of mildly trying to put it all into a document that I can hopefully circulate widely. So your listeners are going to be the first to hear the public release of this conclusion and I'll, I can back it up with science.
Alex Pierson	Very ominous.
Dr. Byram Bridle	So this is what it is. The sars coronavirus 2 has a spike protein on its surface. That spike protein is what it allows it to infect our bodies, that is why we've been using the spike protein in our vaccines. The vaccines we're using get our cells in our body to manufacture that protein. If we can mount an immune response against that protein, in theory, we can prevent this virus from infecting the body. That's the theory behind the vaccine, however, when studying the disease, severe COVID-19 everything that you just described heart problems, lots of problems with cardiovascular system bleeding and clotting is all sociated with severe COVID-19 and looking and doing that research what has been discovered by scientific community is the spike protein on its own is almost entirely responsible for the damage to cardiovascular system if it gets into circulation. Indeed, if you inject the, the purified spike protein into the blood of research animals, they get all kinds of damage to cardiovascular system, it can cross the blood brain barrier and cause damage to the brain. Now at first glance, that doesn't seem too concerning because we're injecting these vaccines into the shoulder muscle. The assumption, all up until now, has been that these vaccines behave like all of our traditional vaccines that they don't go anywhere other than the injection site. So they stay in our shoulder. Some of the protein will go to the local draining lymph node in order to activate the immune system. However, this is where the cutting edge science has come in, and this and this is where it gets scary. Through a request For Information from the Japanese Regulatory agency, myself and several international collaborators have been able to get access to what's called a biodistribution study. It's the first time ever that scientists have been privy to seeing where these messenger RNA vaccines go after vaccination, in other words, is it a safe assumption that it stays in the shoulder muscle? The short answer is absolutely not. It's very disconcerting. The spike protein gets into the blood,

Speaker	Context
	circulates through the blood in individuals over several days post vaccination. It accumulates, once it gets the blood and accumulates in a number of tissues, such as the spleen, the bone marrow, the liver, the adrenal glands. One that's a particular concern for me is it accumulates at quite high concentrations in the ovaries, and, and then also a publication that was just accepted for a scientific paper just accepted for publication that backs this up, looked at 13 young healthcare workers that had received the Moderna vaccine which is the other at messenger RNA based vaccine we have in Canada. They they confirm this, they found the spike protein in circulation, so in the blood of 11 of those 13 healthcare workers that had received the vaccine. What this means is, so we have known for a long time that the spike protein is a pathogenic protein. It is a toxin. It can cause damage in our body if it gets into circulation now we have clear cut evidence that the vaccines that make our bodies are the muscles or the cells in our in our deltoid muscles, right, manufacture this protein that the vaccine itself, plus the protein gets into blood circulation. When in circulation, the spike protein can bind to the receptors that are on our platelets and the cells that line our blood vessels. When that happens, it can do one of two things. It can either cause platelets to clump and that can lead to clotting. That's exactly why we've been seeing clotting disorders associated with these vaccines. It can also lead to bleeding and of course with the hearts involved, it's part actually part of the cardiovascular system. That's why we're seeing heart problems. The protein that can also cross the blood brain barrier and cause neurological damage. That's why also in the fatal case of the blood clots, many times it's seen in the brain and, also of concern is there's also evidence of a of a study this has not yet been accepted for publication, yet, this one, they were trying to show that the antibodies from the vaccine get transferred through breast milk and the idea was this may be a good thing because it would confer some passive protection to babies. However, what they found inadvertently was that the the messenger in vaccines actually get transferred through the breast milk for delivering the vaccine vector itself into infants that are breastfeeding also with this, now we know the spike protein gets into circulation any proteins in the blood will get concentrated in breast milk looking into the adverse event database in the United States, we have found evidence of suckling infants experiencing bleeding disorders in the gastrointestinal tract.

Speaker	**Context**
Alex Pierson	So OK, let me pause you there, cause we've only got about 45 seconds left.
Dr. Byram Bridle	What does mean?
Alex Pierson	I mean the bottom line is
Dr. Byram Bridle	Not sure I'll wrap it up.
Alex Pierson	This will scare a lot of people, this will freak
Dr. Byram Bridle	There's very important this this message, yes.
Alex Pierson	Lot of people out, yeah.
Dr. Byram Bridle	So so this has implications for blood donation right now, Canadian blood services are saying people that who have been vaccinated can donate. We don't want transfer of these pathogenic spike proteins to fragile patients for being transfused with that blood. This has implications for infants that are suckling, and this this has serious implications for people for whom SARS coronavirus 2 is not a high risk pathogen, and this includes all of our children. In short, the conclusion is we made a big mistake. We didn't realize it until now. We thought the spike protein was a great target antigen we never knew the spike protein itself was a toxin and was a pathogenic protein. So by vaccinating people, we are inadvertently inoculating them with a toxin and some people, this gets into circulation and when that happens, in some people, it can cause damage, especially to the cardiovascular system, and I have many other, I don't have time, but many other legitimate questions about the long term safety therefor for this vaccine. For example, with it accumulating in the ovaries, one of my questions is, will we be rendering young people infertile? Some of them infertile? We'll stop there. I know it's heavy hitting but.
Alex Pierson	I have to run up against the clock, I need like an hour when I talk to you because you you have so much information, and of course, you're you're one opinion of many, but you know it's interesting because you have a different look at it and certainly the time will tell on this, but we'll have you on again because I always get an interesting and different perspective from you, doctor. Thank you.

Speaker	**Context**
Dr. Byram Bridle	It was my pleasure. Take care.
Alex Pierson	That is a Doctor Byram Bridle who a lot of you like and like to hear and again, that's his findings. Again, we get lots of different medical opinions that will scare a lot of people, but there are a lot of people, already who don't trust the vaccines, given the speed at which they've come out.

This is Exhibit C referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15 , 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Health Canada Hid Their Concerns About Impurities In COVID-19 Shots From Canadians

DR. BYRAM W. BRIDLE
FEB 24, 2024

The Epoch Times, a media outlet that is not state-funded, released an article yesterday that was updated today. Everyone around the world should read it. You can find it here. The journalist, Noé Chartier, did an excellent job writing a well-balanced, objective, and factual account. I do not have much to add.

For those who might not be able to access the article, here is a summary:

It has been shown that the modified RNA COVID-10 shots are contaminated with bacterial DNA, which, in and of itself, is a serious concern. A more recent finding highlighted additional contaminants that nobody had predicted. The modified RNAs can get mis-read during the process of cells translating them into the intended spike protein from SARS-CoV-2. The result is that up to 8% of the proteins that an injected person's cells make from the modRNAs are unintended, novel foreign proteins that are nothing like the target spike protein.

Laughably, the authors who first highlighted this finding chose the title, "*mRNA vaccines may make unintended proteins, **but there's no evidence of harm**: Alterations that help messenger RNA persist in living cells can trip up protein synthesis*".

The "*no evidence of harm*" component is a standard prerequisite these days to get any concern about modRNA shots published. As per the precautionary principle that was thrown out four years ago, the title should actually state, "*mRNA vaccines may make unintended proteins, **and there's no evidence that these are safe***". The worn-out adage that 'the shots have been given to millions around the world and have 'proven' to be

'safe and effective' can no longer be used as 'evidence of safety'. Why? Because failure to look sufficiently hard for evidence of harms, and downplaying harms that could not be hidden is NOT evidence of safety. When you read the article by *The Epoch Times*, you will see that Health Canada still clings strongly to this misguided philosophical approach to safety.

The part about "*Alterations that help messenger RNA persist in living cells can trip up protein synthesis*" indicates that this unintended and potentially dangerous side-effect is the result of the alterations to natural mRNAs that was recently awarded the Nobel Prize. After all, a Nobel Prize should obviously be awarded for a technology for which unintended side-effects are still being discovered via its premature public rollout around the globe [read with lots of sarcasm].

Health Canada developed concerns about these unintended proteins after learning the real science from my respected colleague, Dr. Jessica Rose, via her Substack article. This article should also be read by everyone since it beautifully describes why these unpredicted contaminating proteins are of legitimate concern.

In short, a scientist at Health Canada that was tasked with investigating this issue agreed that these unintended proteins represent a "high level of impurity". Internal emails from Health Canada were obtained by *The Epoch Times* via a freedom of information request. The communications clearly demonstrate concern within Health Canada about how these recently discovered contaminants (that have been a problem from day one) could negate the authorization of the shots. The result was that Health Canada worked hard to find internal 'fact checkers' willing to downplay the internal concerns. So, the final summary of the investigation hid the most concerning aspects.

Keep in mind that the contaminating off-target proteins are only one in a long and still-growing list of concerns with the modRNA COVID-19 shots. We continue to learn much about the basic mechanisms of action of the technology via the global-scale experiment. So, I call, for the umpteenth time, for a world-wide moratorium on this technology so the research can catch up. Places like the state of Florida in the United States are looking more and more like wise world leaders after having already halted the use of these shots.

For all those people who still claim that the Dr. Jessica Rose's and the rest of the experts that have stood against the tidal wave of the COVID-19 narrative are mere misinformation spreaders, hear this: you no longer need to believe the solid science we have been presenting. Instead, you can look at the burgeoning evidence that your health regulators have been lying to you or hiding important truths from you.

And to top it off, Health Canada keeps trying to take themselves off the hook by dumping full responsibility for assessment and reporting of safety on the big pharma companies. Yes, the same companies that have a keen interest in pushing their products that are profitable to the tune of billions of dollars.

What do the narrative pushers think about health regulatory agencies that not only lie, not only hide concerns, but then obfuscate their responsibility to ensure the safety of citizens by allowing companies that have historical criminal records to 'ensure the safety' of their own products?

I have said this before many times and will say it again… Health regulatory agencies around the world need to be gutted and replaced with people that have three characteristics that too many among the current lot lack:

1. Genuine and relevant subject matter expertise
2. Integrity
3. Courage

Then there needs to be a restored reliance of health regulatory agencies on the financial support of taxpayers rather than big pharma.

Only then will public safety be restored as the demonstrable primary mandate of health regulators. And only then can the explosion of distrust in these agencies be stemmed.

I for one would be more than happy to offer my expertise as a viral immunologist that specializes in vaccinology to assist any government (federal or provincial/state) that is genuinely interested in properly regulating big pharma. Specifically, I would be happy to objectively review their applications, make recommendations for required studies, highlight any concerns, and transparently disseminate the findings to the public. Of

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course, this would need to be done in the context of a job that is protected from negative coercive influences and threats should objective science ever fail to support political or economic narratives.

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106 Comments

Write a comment...

Brian King Brian's Substack Feb 24

Yet another excellent analysis of what's wrong all around us regarding the Covid vaccines. I've been following Dr. Bridle since he first phoned the Toronto radio station to advise them of the Japanese study on the vaccines and Covid. Dr. Bridle is one of the few upstanding, experts now offering his vast knowledge on this topic to authorities who sadly will likely ignore his offer and suggestions of what needs to be done.

LIKE (76) REPLY SHARE

7 replies

Vivien C Buckley Feb 24

I am vaccine injured. All of what I have learned in the last 4 years angers me greatly. The constant stress will do me in I'm sure. I'm sick with worry over my granddaughter whose mother was vaccinated and breast fed. There was no trialling on pregnant women but were pushed regardless. The corruption in peer review, health agencies and governments sickens me. So many very bright and ethical scientists worldwide are alarmed with their findings. Attempts to warn governments is falling on deaf ears. Trust in our institutions is at an all time low for obvious reasons. There's too much corruption, wealth, influence and power in the institutions meant to protect. I'm scared.

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This is Exhibit D referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

JANUARY 3, 2024 | 4 MIN READ

Vaccination Dramatically Lowers Long COVID Risk

Several new studies reveal that getting multiple COVID vaccine doses provides strong protection against lingering symptoms

BY SHANNON HALL

Credit: Jacob Lund/Alamy Stock Photo

Epidemiology

At least 200 million people worldwide have struggled with long COVID: a slew of symptoms that can persist for months or even years after an infection

with SARS-CoV-2, the virus that causes COVID. But research suggests that that number would likely be much higher if not for vaccines.

A growing consensus is emerging that receiving multiple doses of the COVID vaccine before an initial infection can dramatically reduce the risk of long-term symptoms. Although the studies disagree on the exact amount of protection, they show a clear trend: the more shots in your arm before your first bout with COVID, the less likely you are to get long COVID. One meta-analysis of 24 studies published in October, for example, found that people who'd had three doses of the COVID vaccine were 68.7 percent less likely to develop long COVID compared with those who were unvaccinated. "This is really impressive," says Alexandre Marra, a medical researcher at the Albert Einstein Israelite Hospital in Brazil and the lead author of the study. "Booster doses make a difference in long COVID."

It is also a welcome departure from earlierstudies, which suggested that vaccines provided only a modest defense against long COVID. In 2022 Marra's team published a meta-analysis of six studies that found that a single dose of the COVID vaccine reduced the likelihood of long COVID by 30 percent. Now, that protection appears to be much greater.

A study published in November in the *BMJ* found that a single COVID vaccine dose reduced the risk of long COVID by 21 percent, two doses reduced it by 59 percent and three or more doses reduced it by 73 percent. Vaccine effectiveness clearly climbed with each successive dose. "I was surprised that we saw such a clear dose response," says Fredrik Nyberg, an epidemiologist at the University of Gothenburg in Sweden and one of the co-authors of the study. "The more doses you had in your body before your first infection, the better." That lines up with the findings of several new studies, which similarly show this

ladderlike benefit. Marra's October 2023 meta-analysis found that two doses reduced long COVID likelihood by 36.9 percent and three doses reduced it by 68.7 percent. And in a study published last year in the *Journal of the American Medical Association*, other researchers found that the prevalence of long COVID in health care workers dropped from 41.8 percent in unvaccinated participants to 30 percent in those with a single dose, 17.4 percent with two doses and 16 percent with three doses.

These studies were conducted in various countries with differing health care systems, demographics, COVID vaccination uptake and COVID prevalence. As such, Marra notes that the COVID vaccines' effectiveness against long COVID will vary and may not be generalizable to other settings. Still, the consistency of the studies' findings is telling—regardless of their settings, many studies agree that boosters provide potent protection against long COVID. Marra's recent meta-analysis, for example, showed that the prevalence of long COVID in the early years of the pandemic was consistently above 20 percent. Today rates of long COVID have dropped, likely thanks to increased immunity, milder variants and improved treatment. Yet there is still a sharp divide between unvaccinated and vaccinated people. The prevalence of long COVID is currently 11 percent among those who are unvaccinated and 5 percent among those who have had two or more doses of the vaccine. "It is a significant difference for those who are unwilling to take the risk," he says.

The question is why. It could be that these vaccines help prevent severe COVID itself, which is a risk factor for long COVID. But that does not appear to be the whole story—in part because the boosters have also been shown to shield people who had only a mild COVID infection. Unfortunately, the precise mechanisms at play are hard to disentangle because the cause of long COVID itself is still cloaked in mystery. One possibility is that the virus lingers

in the body, finding in various organs—such as the gut or brain—and causing chronic inflammation. Another is that long COVID is an autoimmune disease in which the immune response triggered by the initial infection wages an extended war against the body, causing symptoms long after the initial infection has been cleared.

For both scenarios, boosters give people the upper hand, argues Akiko Iwasaki, an immunologist at Yale University who is co-leading a clinical trial on long COVID. That is because boosters enhance antibodies—increasing both their numbers and their ability to bind to the virus—as well as T and B immune cells that help fight the virus. With both components, "people who take the booster shots have an improved ability to fight off infection," Iwasaki says. And that is key, allowing the booster to quash a growing infection before it spirals out of control. "The more you can prevent the replication and spread of the virus within the body, the less chance the virus has to seed a niche—to establish reservoirs or cause excessive inflammation that leads to autoimmunity," she says.

Although it will take time to pinpoint the exact reason behind vaccines' protective effect against long COVID, many medical experts are hopeful that the new studies will help counter the spread of misinformation and disinformation about the COVID immunizations that has contributed to vaccine hesitancy. But experts also note that while vaccines reduce the risk of long COVID, they do not eradicate it, and protection may wane over time. "Breakthrough infections can still occur, and the dynamic of the virus—including the emergence of new variants—add complexity to the situation," Marra says. As such, he notes that it's important to continue to follow public health guidelines to minimize the impact of COVID, including the risk of long-term symptoms.

SHANNON HALL is an award-winning freelance science journalist based in the Rocky Mountains. She specializes in writing about astronomy, geology and the environment.

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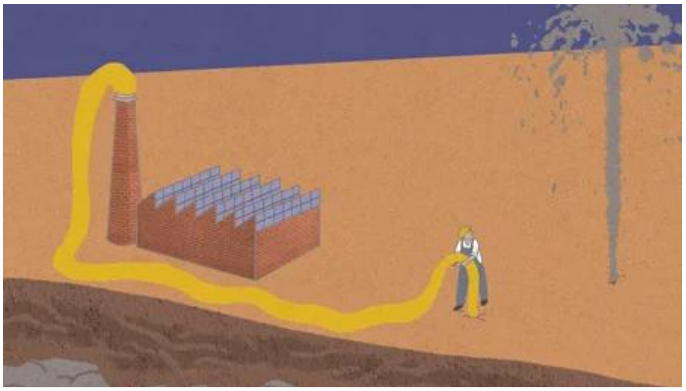

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Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Real-world Effectiveness of BNT162b2 Against Infection and Severe Diseases in Children and Adolescents

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ABSTRACT

Background: The efficacy of the BNT162b2 vaccine in pediatrics was assessed by randomized trials before the Omicron variant's emergence. The long-term durability of vaccine protection in this population during the Omicron period remains limited.

Objective: To assess the effectiveness of BNT162b2 in preventing infection and severe diseases with various strains of the SARS-CoV-2 virus in previously uninfected children and adolescents.

Design: Comparative effectiveness research accounting for underreported vaccination in three study cohorts: adolescents (12 to 20 years) during the Delta phase, children (5 to 11 years) and adolescents (12 to 20 years) during the Omicron phase.

Setting: A national collaboration of pediatric health systems (PEDSnet).

Participants: 77,392 adolescents (45,007 vaccinated) in the Delta phase, 111,539 children (50,398 vaccinated) and 56,080 adolescents (21,180 vaccinated) in the Omicron period.

Exposures: First dose of the BNT162b2 vaccine vs. no receipt of COVID-19 vaccine.

Measurements: Outcomes of interest include documented infection, COVID-19 illness severity, admission to an intensive care unit (ICU), and cardiac complications. The effectiveness was reported as (1-relative risk)*100% with confounders balanced via propensity score stratification.

Results: During the Delta period, the estimated effectiveness of BNT162b2 vaccine was 98.4% (95% CI, 98.1 to 98.7) against documented infection among adolescents, with no significant waning after receipt of the first dose. An analysis of cardiac complications did not find an increased risk after vaccination. During the Omicron period, the effectiveness against documented infection among children was estimated to be 74.3% (95% CI, 72.2 to 76.2). Higher levels of effectiveness were observed against moderate or severe COVID-19 (75.5%, 95% CI, 69.0 to 81.0) and ICU admission with COVID-19 (84.9%, 95% CI, 64.8 to 93.5). Among adolescents, the effectiveness against documented Omicron infection was 85.5% (95% CI, 83.8 to 87.1), with 84.8% (95% CI, 77.3 to 89.9) against moderate or severe COVID-19, and 91.5% (95% CI, 69.5 to 97.6)) against ICU admission with COVID-19. The effectiveness of the BNT162b2 vaccine against the Omicron variant declined after 4 months following the first dose and then stabilized. The analysis revealed a lower risk of cardiac complications in the vaccinated group during the Omicron variant period.

Limitations: Observational study design and potentially undocumented infection.

Conclusions: Our study suggests that BNT162b2 was effective for various COVID-19-related outcomes in children and adolescents during the Delta and Omicron periods, and there is some evidence of waning effectiveness over time.

Primary Funding Source: National Institutes of Health

INTRODUCTION

The Food and Drug Administration (FDA) expanded the emergency use authorization of the BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech) to 12-15-year-olds on May 10, 2021, and to 5-11-year-olds on October 29, 2021. As of April 5, 2023, the Centers for Disease Control and Prevention (CDC) reports indicate that 40% of U.S. children aged 5-to-11-year-olds and 72% of adolescents aged 12-to-18-year-olds had received at least one dose of the vaccine. The emergence of the Omicron variant (B.1.1.529)

and its subvariants in early 2022 led to a new surge in COVID-19 cases worldwide (1). The randomized trials of the BNT162b2 vaccine which demonstrated high efficacy of 2 doses against COVID-19 (100% and 91% among those aged 12-15 and 5-11 years, respectively) were conducted before the emergence of the Omicron variant (2,3).

Several observational studies have been conducted to investigate the effectiveness of vaccination in real-world settings (4–8). However, prior studies have had limited follow-up periods, covering the Delta variant or earlier subvariants of Omicron periods only. Studies evaluating the Omicron variant have only assessed the short-term effects of the vaccine, with only one study involving children evaluating the effect beyond 3 months (9). There is limited information on the long-term durability of vaccine protection during the Omicron period. Few existing studies on U.S. pediatric populations have covered both hospitalized patients and those with mild or asymptomatic conditions. Furthermore, while studies have acknowledged limitations due to misclassification in vaccination status in real-world effectiveness studies, none have rigorously evaluated the impacts of such misclassification nor accounted for the potential bias it may introduce.

To address these gaps in our knowledge of the pediatric effectiveness of SARS-CoV-2 vaccination, we designed this study to assess the real-world effectiveness of BNT162b2 among children and adolescents during the Delta and Omicron variant-predominant periods using electronic health record (EHR) data from a national network of U.S. pediatric medical centers. Our study used a novel comparative effectiveness research (CER) method and adjusted for underreporting issues in vaccination status and has several attractive features that strengthen reliability of our inference. First, it is the largest study to date in the U.S. estimating vaccine effectiveness in children and adolescents, covering a broad spectrum of the U.S. pediatric population. Second, the study examined the effectiveness against infection over a longer follow-up period than any previous study, enabling evaluation of the durability of vaccine protection. Third, the study included a diverse representation of U.S. pediatric populations from primary care, specialty care, emergency department, testing centers, and inpatient settings. Fourth, the study was the first to account for the incomplete capture of vaccination status by health systems in the U.S. Finally, in addition to infection and severe disease endpoints, we also studied the effect of vaccination on the incidence of myocarditis, pericarditis or multisystem inflammatory syndrome (MIS) to assess the effect of vaccination relative to potential risk of cardiac complications.

METHODS

DATA SOURCES

This study used EHR data from PEDSnet (10), which is a national collaboration of pediatric health systems that share EHR data, conduct research, and improve outcomes together. Participating institutions in this study included: Children's Hospital of Philadelphia, Cincinnati Children's Hospital Medical Center, Children's Hospital Colorado, Ann & Robert H. Lurie Children's Hospital of Chicago, Nationwide Children's Hospital, Nemours Children's Health System (inclusive of the Delaware and Florida health system), Seattle Children's Hospital, and Stanford Children's Health. Data were extracted from the PEDSnet COVID-19 Database Version Week 141 (11). A detailed description of EHR data is available in Section S1 of the Supplementary Appendix.

SPECIFICATION OF HYPOTHETICAL TRIALS AND CER STUDIES

Hypothetical randomized controlled trials (RCT) were specified to guide the design of observational studies to assess the real-world effectiveness of treatments (12). We designed and conducted CER studies to investigate the effectiveness of the BNT162b2 vaccine in preventing infection with various strains of the SARS-CoV-2 virus in children and adolescents in the United States. The three study cohorts focused on documented SARS-CoV-2 infection and outcomes in:

- ***Study cohort 1*** (Delta study in adolescents): adolescents aged 12 to 20 years during the period when the Delta variant was prevalent from July 1, 2021, to November 30, 2021.
- ***Study cohort 2*** (Omicron study in children): children aged 5 to 11 years during the period when the Omicron variant was prevalent from January 1, 2022, to November 30, 2022.
- ***Study cohort 3*** (Omicron study in adolescents): adolescents aged 12 to 20 years during the period when the Omicron variant was prevalent from January 1, 2022, to November 30, 2022.

The design of hypothetical trials and implementation procedures in real-world data are summarized in Table S1 of the Supplementary Appendix.

Eligibility criteria included age of 5 to 11 years for children or 12 to 20 years for adolescents at the start of the study period and no previous COVID-19 vaccination or documented SARS-CoV-2 infection. Additionally, participants were required to have a prior encounter (including telephone or telehealth encounters) within 18 months of cohort entrance to ensure that they had an ongoing interaction with the health system.

The intervention of interest was vaccination, in comparison with no receipt of any type of COVID-19 vaccine. Since the BNT162b2 vaccine covered more than 85% of documented vaccinations among children and adolescents in the PEDSnet database, in this study we focused primarily on studying the effectiveness of this vaccine, although the supplementary appendix reports a sensitivity analysis investigating all types of reported COVID-19 assessed in the U.S., with 85.7% BNT162b2, 1.9% mRNA-1273, and 12.3% unspecified COVID-19 vaccine.

In this CER study using real-world data, the cohort entrance date for the intervention group was defined as the date of the first dose of the BNT162b2 vaccine, while for the comparator group, it was a randomly selected date from visits, chosen to ensure the distribution of index dates for the control group matched the distribution of index dates for the vaccination group to control for time effects. The risk period for the study began 28 days after the index date such that infections within 28 days were excluded.

Randomized trials achieve balance across potential confounders by randomly allocating the treatment to intervention and comparator groups. In our study, we attempted to balance the intervention and comparator groups by adjusting for a large number of measured confounders collected prior to cohort entry using propensity score stratification (13). We built the propensity score model based on demographic factors including age, sex, race/ethnicity, clinical factors including obesity status, a baseline chronic condition indicator as defined by the Pediatric Medical Complexity Algorithm (PMCA) (14), and a list of pre-existing chronic conditions, and healthcare utilization factors collected prior to the cohort entry including the number of inpatients, outpatients, ED visits, unique mediations, and the number of negative COVID-19 tests. We stratified the patients into propensity score quintiles based on these factors. See Table S3 in the Supplementary Appendix for detailed definitions of study variables.

The four COVID-19 outcomes of interest were: documented SARS-CoV-2 infection, mild COVID-19, moderate/severe COVID-19, and ICU admission with COVID-19. We did not evaluate death from COVID-19 as it was too rare among children and adolescents to study quantitatively. In our study, SARS-CoV-2 infections were defined by and occurrence of positive polymerase-chain-reaction (PCR), serology, or antigen tests or diagnoses of COVID-19, post-acute sequelae of SARS-CoV-2 (PASC), or multisystem inflammatory syndrome in children (MIS-C) regardless of the presence of symptoms. Classification of mild, moderate, or severe COVID-19 infections was defined based on the symptoms and health conditions diagnosed from 7 days prior to 13 days post the date of a documented COVID-19 infection as in Forrest et al. (2022) (15). ICU admission with COVID-19 was defined by any ICU visit 7 days prior to 13 days after documented SARS-CoV-2 infection. Additionally, we considered the clinical outcomes of cardiac complications identified as incidence of myocarditis, pericarditis or MIS to allow for a

comprehensive capture of potential cardiac complications after infection and evaluate the effect of the BNT162b2 vaccine in terms of cardiac risks.

STATISTICAL ANALYSIS

We evaluated covariate balancing after propensity score stratification by plotting the standardized mean differences (SMD) between variable values for the vaccinated and unvaccinated groups, with a difference of 0.1 or less indicating an acceptable balance. We used Poisson regression to estimate the relative risk between two treatment arms for the risk of each outcome while adjusting for different follow-up lengths among participants. Since immunization records are often captured and stored across multiple disconnected sources, resulting in incomplete vaccination records in patients' EHRs, we mitigated the potential bias arising from this underreporting issue by incorporating an integration likelihood(16) of the Poisson regression with a pre-specified range of misclassification rates. The vaccine effectiveness was defined as 100*(1-relative risk). The details of statistical methods are described in Section S2 of the Supplementary Appendix.

We conducted secondary analyses stratified by 2-month intervals since receipt of vaccination to investigate the durability of vaccine protection. Subgroup analyses were also performed to investigate differences in vaccine protection according to age groups (5-to-8, 9-to-11, 12-to-15, 16-to-20).

SENSITIVITY ANALYSES

Extensive sensitivity analyses were conducted to evaluate the robustness of the research findings; see Supplementary Appendix Sections S6 – S15, S19 for the impacts of cohort design. In scenarios in which any categorical covariates were unbalanced (with a standardized mean difference>0.1), we included a sensitivity analysis excluding participants in that category. A sensitivity analysis defining the risk period with no waiting period after the index date was conducted. To assess whether population heterogeneity, established by applying eligibility criteria that required a prior encounter within 18 months of cohort entry, could influence our vaccine effectiveness estimates, we conducted sensitivity analyses within a more restricted time window. A sensitivity analysis was conducted to compare the proposed method to a sequential target trial emulation pipeline not accounting for underreporting issues to assess the robustness of findings. Since the proportion of patients entered with ED visits is relatively low in the vaccinated cohort compared to the unvaccinated, we conducted a sensitivity analysis excluding all participants who entered the cohort during an ED visit. Residual study bias from unmeasured and systematic sources can still exist in observational studies after controlling for measured confounders; thus, we conducted negative control outcome experiments (13,17,18) in which the null hypothesis of no effect was believed to be true using 40 negative control outcomes pre-specified by pediatric physicians. The empirical null distribution and calibrated effectiveness were reported as sensitivity analyses. The relative risks for cardiac complications defined by myocarditis or pericarditis (excluding MIS) were estimated. We also reported the estimated vaccine effectiveness from all brands of COVID-19 vaccines. Given the prolonged presence of the Omicron variant and the emergence of several sub-variants, we conducted a secondary analysis to assess the vaccine effectiveness related to sub-variants of Omicron.

MISSINGNESS IN VACCINE RECORDS

Vaccine status may be missing for individuals whose vaccine doses were administered by a site outside of the PEDSnet network care delivery sites. It is likely that patients recorded as vaccinated in the EHR are true positives, so specificity could be very high, but sensitivity would be reduced by undocumented vaccinations (false negatives). To account for potential bias from the underreporting issue in vaccination status, a range of possible sensitivities based on our prior study was pre-specified for each study. The sensitivity range was considered to be 0.8 to 1 for the study involving children and 0.7 to 0.9 for the studies involving adolescents. By accounting for the underreporting and specifying a range of sensitivity, the study aimed to minimize the impact of bias caused by the underreporting in the estimation of the

effectiveness of the COVID-19 vaccine among children and adolescents. The details of statistical methods are described in Section S2 of the Supplementary Appendix.

To further evaluate the robustness of statistical methods we used to account for underreporting, we varied our CER method by considering alternative methods for bias correction, including the naive method (without adjusting for underreporting), using different ranges of misclassification rates, and using a fully Bayesian method (19). To evaluate the impact of differential misclassification on effectiveness estimates, we conducted sensitivity analyses simulating vaccination status according to various differential misclassification scenarios. Results from these sensitivity analyses are summarized in Sections S16-18 of the Supplementary Appendix.

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RESULTS

STUDY POPULATION

A total of 77,392 adolescents (45,007 vaccinated) within the PEDSnet network were identified to study the effectiveness of vaccination against Delta infection and severe outcomes (see Table 1 for baseline characteristics). 111,539 children (50,398 vaccinated) and 56,080 adolescents (21,180 vaccinated) were included in the cohort to study the effectiveness of vaccination against the Omicron infections (see Table 2 for baseline characteristics). The vaccinated and unvaccinated groups had a slightly unbalanced distribution of testing rates before cohort entry across all three cohorts. After propensity-score stratification, all covariates were well balanced between vaccinated and unvaccinated groups with an SMD smaller than 0.1 in the Omicron study involving children (Figure S13) and involving adolescents (Figure S14). In the study evaluating vaccine effectiveness for adolescents during the Delta period, one site remained unbalanced after propensity-score stratification with an SMD larger than 0.1, and thus a sensitivity analysis was conducted by excluding participants from this site which gave consistent results with the primary analysis (see Figure S12 and Section S6 in Supplementary Appendix). Additional characteristics of study cohorts including additional medical conditions, deaths, follow-up durations were summarized in Section S5 of Supplementary Appendix.

VACCINE EFFECTIVENESS

Table 3 summarizes the estimated vaccine effectiveness in three study cohorts and Figure 2 shows the durability of protection. The vaccine effectiveness was estimated to be 98.4% (95% CI, 98.1 to 98.7) among adolescents in the Delta period, 74.3% (95% CI, 72.2 to 76.2) against documented infection among children in the Omicron period, and 85.5% (95% CI, 83.8 to 87.1) among adolescents in the Omicron period. During the Delta period, the vaccine effectiveness against documented infection remained stable throughout the follow-up period of the study. After 4 months following the first dose, vaccine effectiveness against documented infection with Omicron declined from 82.3% (95% CI, 77.9 to 85.8) to 70.6% (95% CI, 65.9 to 74.6) among children, and from 91.3% (95% CI, 87.6 to 94.0) to 82.9% (95% CI, 79.0 to 86.1) among adolescents. Although vaccine effectiveness against documented infection stabilized after this initial decline, the corresponding confidence intervals were much wider indicating higher levels of uncertainty.

VACCINE EFFECTIVENESS: SEVERE ILLNESS AND COMPLICATIONS

During the Delta period, the vaccine was found to have high effectiveness against severe infections. The estimated relative risk the vaccine on cardiac complications was 1.22 (95% CI, 0.34 to 4.35). The estimated vaccine effectiveness against the Omicron variant in children was 73.5% (95% CI, 69.2 to 77.1) against mild COVID-19, 75.5% (95% CI, 69.0 to 81.0) against moderate or severe COVID-19, and 84.9% (95% CI, 64.8 to 93.5) against ICU admission with COVID-19. The estimated relative risk the vaccine on cardiac complications was 0.28 (95% CI, 0.08 to 0.95). In the Omicron study in adolescents, the vaccine effectiveness was estimated to be 87.0% (95% CI, 83.5 to 89.8) against mild COVID-19, 84.8% (95% CI, 77.3 to 89.9) against moderate or severe COVID-19, and 91.5% (95% CI, 69.5 to 97.6) against ICU admission with COVID-19. The estimated relative risk of the vaccine on cardiac complications was 0.10 (95% CI, 0.02 to 0.57) in this cohort.

SENSITIVITY ANALYSES AND ADDRESSING MISCLASSIFICATION BIAS

Section S9 presents the vaccine effectiveness against various Omicron sub-variants. The effectiveness against sub-variants BA.1, BA.2, BA.4, and BA.5 aligns with our primary findings, while the vaccine's effectiveness appears to be lower against the BQ.1, XBB, and subsequent sub-variants. Section S14 presents negative control experiments of three study cohorts using 40 negative control outcomes. After accounting for systematic error through calibration using negative control outcomes, our findings indicate a slight shift in point estimates accompanied by wider confidence intervals. This suggests the presence of a minor degree of systematic error, as well as additional uncertainty characterized by the estimated distribution derived from the negative control outcomes. Section S19 summarizes the comparative results to a sequential target trial emulation not accounting for underreporting issues of vaccination, which indicated reasonably consistent findings.

Section S16 shows effectiveness estimated from the naive method and proposed CER method with different ranges of sensitivity of vaccination status captured by EHR. The comparison results indicated that the vaccine effectiveness was reasonably consistent across different sensitivity ranges, suggesting that our primary analysis was robust to changes in the range of sensitivity considered. Section S17 shows the comparative results to a fully Bayesian method indicating nearly identical results. Section S18 shows sensitivity analyses on differential misclassifications which demonstrates the novel CER method corrects the bias even when the non-differential misclassification assumption does not hold.

DISCUSSION

We estimated the effectiveness of BNT162b2 vaccines for the prevention of documented COVID-19 infections and severe disease in a national network of pediatric health systems in the U.S. for three study cohorts. During the period of time where the Delta variant was dominant, the BNT162b2 vaccine in adolescents was associated with strong protection with effectiveness higher than 95% and with little evidence of waning during the follow-up period. Our findings against the Delta infection among adolescents are consistent with vaccine efficacy observed in the BNT162b2 clinical trial involving adolescents between 12 and 15 years of age, which demonstrated vaccine efficacy of 100% (95% CI, 75.3 to 100) against confirmed COVID-19 infection(2). Our estimates of vaccine effectiveness against severe diseases are consistent with a case-control study based on test-negative design, which found an effectiveness of 94% (95% CI, 90 to 96) against hospitalization and 98% (95% CI, 93 to 99) against ICU admission(7).

In our study, during the period of time where the Omicron variant was dominant, the estimated vaccine effectiveness was approximately 70% for children and 85% for adolescents. The estimated protection decreased by roughly 10% around four months from the first dose and slightly waned over time. Previous studies have shown vaccine effectiveness against Omicron infection, ranging from 20 to 80% among children and adolescents. An analysis using a test-negative design in Scotland during Omicron period

revealed a vaccine effectiveness of 81.2% (95% CI, 77.7 to 84.2) for adolescents aged 12 to 15 years, and 65.5% (95% CI, 56.0 to 73.0) for those aged 16 to 17 years, 2-5 weeks post full vaccination. This effectiveness decreased to 43.3% (95% CI, 30.0 to 54.2) and 8.9% (95% CI, -19.1 to 30.3) after 10-13 weeks, respectively(20). With a test-negative design, data from U.S. pharmacy-based, drive-through SARS-CoV-2 testing sites indicated that the estimated vaccine effectiveness for children aged 5 to 11 years was 60.1% within 2 to 4 weeks following the second dose and declined to 28.9% during the second month post-vaccination. For adolescents aged 12 to 15 years, the effectiveness was 59.5% within 2 to 4 weeks after the second dose and dropped to 16.6% during the subsequent month (21). Another test-negative design analysis in U.S. indicated that in children aged 5–11 years, the effectiveness was 59.9% (95% CI 58.5 to 61.2) at 1 month, 33.7% (32.6 to 34.8) at 4 months, and 14.9% (95% CI 12.3 to 17.5) at 10 months following the first dose(22). A retrospective study among Italy children aged 5-to-11 years shown the vaccine effectiveness to be 29.4% (95% CI 28.5 to 30.2) against documented infection and 41.1% (95% CI 22.2 to 55.4) against severe COVID-19(23). A Singapore study founded that in fully vaccinated children aged 5-to-11 years, vaccine effectiveness was 65.3% (95% CI, 62.0 to 68.3) against PCR-confirmed SARS-CoV-2 infection and 82.7% (95% CI, 74.8 to 88.2) against COVID-19-related hospitalization (24). Additionally, we found that the vaccine effectiveness is substantially lower against the later sub-variants of Omicron including BQ.1, XBB. However, it remains unclear whether this is a true sub-variant effect or evidence of further waning over time. Continued research is desirable to understand the vaccine effectiveness on future sub-variants and its potential waning effects.

This study did not identify a statistically significant elevated risk of cardiac complications among vaccinated adolescents during the Delta variant period, and it even demonstrated a lower risk in the vaccinated group during the Omicron variant period. This finding might seem unexpected, given that cases of myocarditis and pericarditis after mRNA COVID-19 vaccines received significant attention(25–27). However, it is essential to note that previous studies indicated a much higher risk of myocarditis or pericarditis following a documented COVID-19 infection in the pediatric population(28), with one paper finding 36.8 times higher risk (95% CI 25.0 to 48.6) in less than 16 years old after COVID-19(29), and that myocarditis is a common symptom for patients diagnosed with MIS(30,31). It is possible cases of myocarditis, pericarditis or MIS occurred after undocumented COVID-19 infections. For example, 31.5% of occurrences of MIS did not have COVID-19 infections documented in the PEDSnet EHR database. Also note that during this period, especially the Omicron period, positive tests may have been from at-home tests or otherwise outside the system. Further assessment of cardiac complications after vaccination and COVID-19 is warranted to help provide a more complete picture of risk or benefit during a changing pandemic.

Our study has several strengths. First, we used a national network of academic medical centers that covered a diverse cohort being more representative of the general pediatric population, provided a robust sample size, and allowed for multiple subgroup analyses and detection of rare outcomes. Second, the richness of these EHR data allowed us to investigate the effectiveness against infection of different levels of severity as well as adjust for a broad set of confounders. Third, we conducted the negative control outcome experiments to assess the potential residual bias due to unmeasured confounders and other potential sources of systematic bias in the data. These experiments revealed a small amount of systematic error but with excessive uncertainty across different negative control outcomes, leading to wider confidence intervals of our estimated effectiveness that honestly reflect the impacts of unmeasured confounding and other potential sources of residual biases(32). Finally, to the best of our knowledge, this is the first real-world effectiveness study evaluating COVID-19 vaccines against infection and severe outcomes that explicitly handle the underreporting in vaccination.

Our study also has several potential limitations. First, effectiveness was investigated against documented infection in a cohort without previous infection, while the potential inclusion of patients with undocumented infections exists. Nevertheless, if this potential lack of data is evenly distributed across

treatment arms, it could potentially attenuate the true vaccine effectiveness, thereby making our analysis more conservative in its estimations. Our inclusion of previous negative COVID-19 tests as a confounder aims to balance the probability of testing between treatment arms which could partially adjust for this factor. Moreover, the increasing availability of at-home rapid antigen testing kits over time could have further reduced the testing frequency captured by EHR. However, severe cases who test positive through home kits typically seek medical care and report their results to hospitals. This would lead to a better capture of severe infection in our database and more reliable vaccine effectiveness estimates. Baseline confounders were balanced between vaccinated and unvaccinated groups, which should adjust for between-cohort differences in exposure risks and risks of severe infections. Second, as in any observational study, assignment to the vaccine group was non-random and the validity of the results could be impacted by unmeasured confounders. To evaluate the impact of unmeasured confounders and residual bias, we conducted negative control experiments that quantified the robustness of our results.

Third, in this study, patients who had received vaccinations prior to the start of the study period were excluded. Due to missing vaccine records, some patients who had previously been vaccinated may have still entered the cohort, particularly in the unvaccinated group. However, the CER method used in this study adjusted for potential bias resulting from unrecorded vaccinated patients which could also reduce the bias resulting from this issue. Finally, in the Omicron study involving adolescents, the cohort included adolescents who had their first vaccine after January 1, 2022. Since the use of BNT162b2 vaccines was authorized in adolescents aged 12-15 years on May 10, 2021, this cohort may represent a population with late vaccines which reduces the generalizability of the findings.

Although this study provides evidence of a slight waning of vaccine effectiveness 4 months following the first dose against Omicron infection and the effectiveness is stabilized after 4 months, waning can be impacted by vaccines during the follow-up period and other factors. Patients who got boosters during the follow-up period were not excluded from the study. A sensitivity analysis evaluating the durability of two-dose vaccine effectiveness considering the third dose as censoring did not suggest a significantly different conclusion. A future study is warranted to investigate the effect of booster vaccination among children and adolescents. Furthermore, despite the recognized risk of myocarditis associated with COVID-19 vaccines in young men and teen boys, the study reveals a lower relative risk of myocarditis, pericarditis, or MIS in vaccinated groups which may be partially explained by reduced likelihood of infection during the study period(33).

In summary, this study involving national pediatric cohorts in the U.S. estimates moderate effectiveness of the BNT162b2 vaccine for preventing infection and severe diseases of the SARS-CoV-2 Omicron variant, and high effectiveness against the Delta variant. This study reveals a low risk of cardiac complications among children and adolescents who were vaccinated during the Omicron period, which was statistically insignificant in the Delta period. Our assessment of vaccine effectiveness across diverse outcomes underscores the vaccine's pivotal role in reducing SARS-CoV-2 transmission, minimizing COVID-19 related sick leaves, and alleviating economic burdens during the pandemic. This study significantly contributes to our knowledge of the BNT162b2 vaccine in the U.S. pediatric population using a rigorously designed CER method accounting for the incomplete capture of vaccination status in EHR data in the U.S.

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Figure 1. Selection of participants for the three study cohorts evaluating the effectiveness of the BNT162b2 vaccine in preventing infection with SARS-CoV-2 in (*Study cohort 1*) adolescents aged 12-20 years during the period when the Delta variant was prevalent, (*Study cohort 2*) children aged 5 to 11 years and (*Study cohort 3*) adolescents aged 12 to 20 years during the period when the Omicron variant was prevalent.

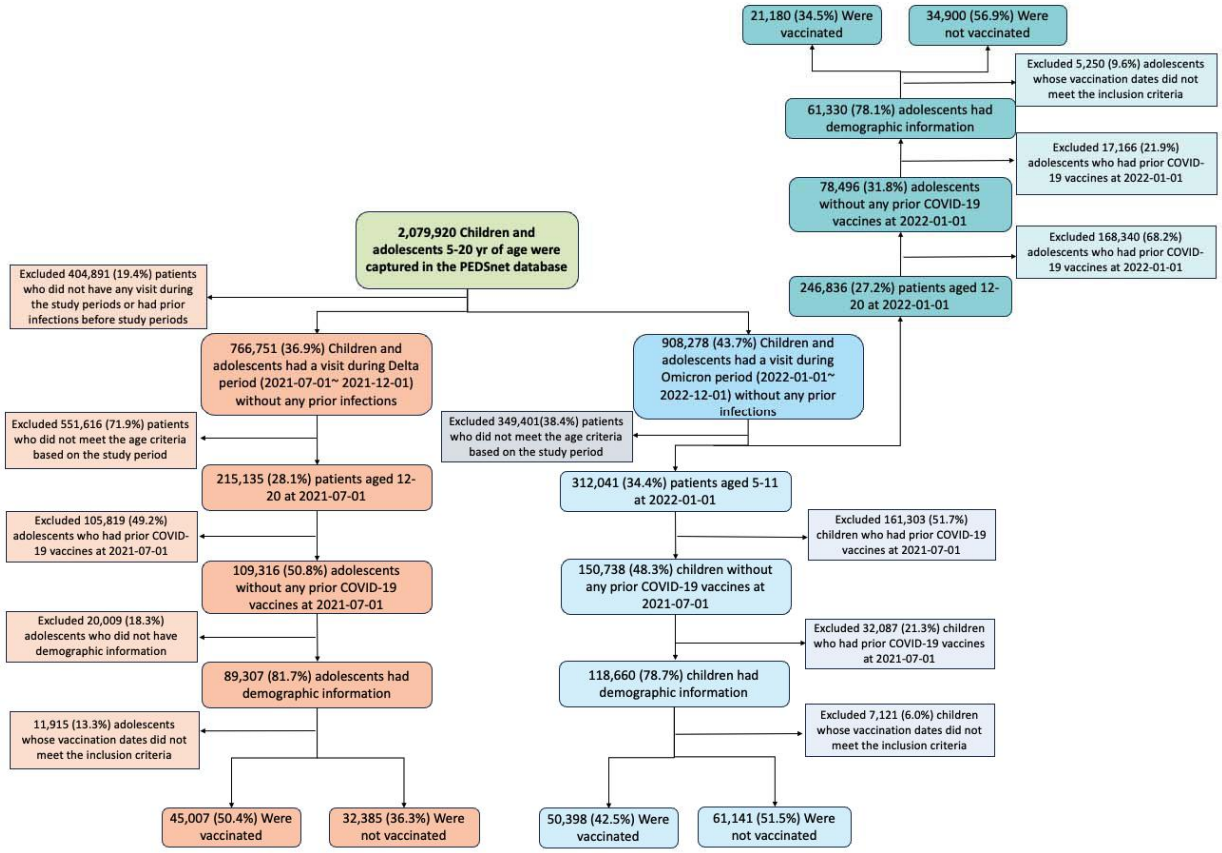


Table 1. Baseline characteristics of adolescents 12 to 20 years of age in the study of the effectiveness of the BNT162b2 vaccine in preventing infection and severe diseases with SARS-CoV-2 during the period when the Delta variant was prevalent.

	Delta study in adolescents		
	Vaccinated (N=45,007)	**Unvaccinated (N=32,385)**	**Overall (N=77,392)**
Age			
Median [Q1, Q3]	14 [13, 16]	15 [13, 17]	15 [13, 16]
Distribution			
12	8922 (19.8%)	4926 (15.2%)	13848 (17.9%)
13	8099 (18.0%)	4864 (15.0%)	12963 (16.7%)
14	8037 (17.9%)	4923 (15.2%)	12960 (16.7%)
15	7311 (16.2%)	4749 (14.7%)	12060 (15.6%)
16	5450 (12.1%)	4546 (14.0%)	9996 (12.9%)
17	4076 (9.1%)	3991 (12.3%)	8067 (10.4%)
18	1674 (3.7%)	2075 (6.4%)	3749 (4.8%)
19	942 (2.1%)	1461 (4.5%)	2403 (3.1%)
20	496 (1.1%)	850 (2.6%)	1346 (1.7%)
Gender			
Female	23,589 (52.4%)	16,500 (50.9%)	40,089 (51.8%)
Male	21,416 (47.6%)	15,880 (49.0%)	37,296 (48.2%)
Ethnicity			
White	16,446 (50.8%)	17,964 (39.9%)	34,410 (44.5%)
Black/AA	6,019 (18.6%)	12,012 (26.7%)	18,031 (23.3%)
Hispanic	4,925 (15.2%)	9,629 (21.4%)	14,554 (18.8%)
Other/Unknown	4,995 (15.4%)	5,402 (12.0%)	10,397 (13.4%)
Hospital			
A	5,424 (12.1%)	7,385 (22.8%)	12,809 (16.6%)
B	12,884 (28.6%)	5,216 (16.1%)	18,100 (23.4%)
C	6,333 (14.1%)	3,457 (10.7%)	9,790 (12.6%)
D	1,723 (3.8%)	914 (2.8%)	2,637 (3.4%)
E	4,369 (9.7%)	6,063 (18.7%)	10,432 (13.5%)
F	12,831 (28.5%)	3,409 (10.5%)	16,240 (21.0%)
G	1,430 (3.2%)	1,457 (4.5%)	2,887 (3.7%)
H	13 (0.0%)	4,484 (13.8%)	4,497 (5.8%)
Entry time			
07/2021 - 09/2021	38,335 (85.2%)	24,509 (75.7%)	62,844 (81.2%)
10/2021 - 11/2021	6,672 (14.8%)	7,876 (24.3%)	14,548 (18.8%)

Obesity			
0	28,029 (62.3%)	22,479 (69.4%)	50,508 (65.3%)
1	16,978 (37.7%)	9,906 (30.6%)	26,884 (34.7%)
PMCA*			
0	25,634 (57.0%)	19,916 (61.5%)	45,550 (58.9%)
1	10,915 (24.3%)	6,417 (19.8%)	17,332 (22.4%)
2	8,458 (18.8%)	6,052 (18.7%)	14,510 (18.7%)
Negative tests prior entry			
0	1,330 (3.0%)	2,888 (8.9%)	4,218 (5.5%)
1	34,272 (76.1%)	16,299 (50.3%)	50,571 (65.3%)
2	7,388 (16.4%)	9,739 (30.1%)	17,127 (22.1%)
>=3	2,017 (4.5%)	3,459 (10.7%)	5,476 (7.1%)

*PMCA: Pediatric Medical Complexity Algorithm

Table 2. Baseline characteristics of children 5 to 11 and adolescents 12 to 20 years of age in the study of the effectiveness of the BNT162b2 vaccine in preventing infection and severe diseases with SARS-CoV-2 during periods when Omicron variant was prevalent.

	Omicron study in children			*Omicron study in adolescents*		
	Vaccinated (N=50,398)	**Unvaccinated (N=61,141)**	**Overall (N=111,539)**	**Vaccinated (N=21,180)**	**Unvaccinated (N=34,900)**	**Overall (N=56,080)**
Age						
Median [Q1, Q3]	8 [6, 10]	7 [6, 9]	8 [6, 10]	14 [13, 16]	15 [13, 17]	15 [13, 17]
Distribution						
5	8,165 (16.2%)	13,321 (21.8%)	21,486 (19.3%)			
6	7,447 (14.8%)	11,314 (18.5%)	18,761 (16.8%)			
7	7,090 (14.1%)	9,151 (15.0%)	16,241 (14.6%)			
8	7,028 (13.9%)	7,922 (13.0%)	14,950 (13.4%)			
9	6,773 (13.4%)	7,085 (11.6%)	13,858 (12.4%)			
10	7,011 (13.9%)	6,434 (10.5%)	13,445 (12.1%)			
11	6,884 (13.7%)	5,914 (9.7%)	12,798 (11.5%)			
12				4754 (22.4%)	5760 (16.5%)	10514 (18.7%)
13				3421 (16.2%)	5520 (15.8%)	8941 (15.9%)
14				3338 (15.8%)	5299 (15.2%)	8637 (15.4%)
15				3123 (14.7%)	5315 (15.2%)	8438 (15.0%)
16				2634 (12.4%)	4944 (14.2%)	7578 (13.5%)
17				2201 (10.4%)	3836 (11.0%)	6037 (10.8%)
18				986 (4.7%)	2114 (6.1%)	3100 (5.5%)
19				475 (2.2%)	1400 (4.0%)	1875 (3.3%)
20				248 (1.2%)	712 (2.0%)	960 (1.7%)
Gender						
Female	23,962 (47.5%)	28,669 (46.9%)	52,631 (47.2%)	11,402 (53.8%)	17,954 (51.4%)	29,356 (52.3%)
Male	26,436 (52.5%)	32,468 (53.1%)	58,904 (52.8%)	9,775 (46.2%)	16,939 (48.5%)	26,714 (47.6%)
Ethnicity						
White	14,399 (28.6%)	24,644 (40.3%)	39,043 (35.0%)	16,240 (46.5%)	6,836 (32.3%)	23,076 (41.1%)
Black/AA	13,711 (27.2%)	13,733 (22.5%)	27,444 (24.6%)	6,154 (17.6%)	6,157 (29.1%)	12,311 (22.0%)
Hispanic	12,119 (24.0%)	12,781 (20.9%)	24,900 (22.3%)	6,287 (18.0%)	3,784 (17.9%)	10,071 (18.0%)
Other/Unknown	10,169 (20.2%)	9,983 (16.3%)	20,152 (18.1%)	6,219 (17.8%)	4,403 (20.8%)	10,622 (18.9%)
Hospital						
A	5,019 (10.0%)	9,266 (15.2%)	14,285 (12.8%)	2,131 (10.1%)	5,183 (14.9%)	7,314 (13.0%)
B	15,229 (30.2%)	13,168 (21.5%)	28,397 (25.5%)	6,397 (30.2%)	6,556 (18.8%)	12,953 (23.1%)
C	5,482 (10.9%)	7,409 (12.1%)	12,891 (11.6%)	1,719 (8.1%)	4,075 (11.7%)	5,794 (10.3%)
D	4,766 (9.5%)	2,878 (4.7%)	7,644 (6.9%)	678 (3.2%)	1,337 (3.8%)	2,015 (3.6%)

E	5,843 (11.6%)	10,551 (17.3%)	16,394 (14.7%)	2,047 (9.7%)	4,263 (12.2%)	6,310 (11.3%)
F	9,786 (19.4%)	11,348 (18.6%)	21,134 (18.9%)	3,563 (16.8%)	5,186 (14.9%)	8,749 (15.6%)
G	1,250 (2.5%)	3,239 (5.3%)	4,489 (4.0%)	622 (2.9%)	2,353 (6.7%)	2,975 (5.3%)
H	3,023 (6.0%)	3,282 (5.4%)	6,305 (5.7%)	4,023 (19.0%)	5,947 (17.0%)	9,970 (17.8%)
Entry time						
01/2022 - 03/2022	37,970 (75.3%)	32,523 (53.2%)	70,493 (63.2%)	14,684 (69.3%)	1,9032 (54.5%)	33,716 (60.1%)
04/2022 - 06/2022	5,882 (11.7%)	11,919 (19.5%)	17,801 (16.0%)	3,344 (15.8%)	7,087 (20.3%)	10,431 (18.6%)
07/2022 - 09/2022	4,994 (9.9%)	10,329 (16.9%)	15,323 (13.7%)	2,206 (10.4%)	5,479 (15.7%)	7,685 (13.7%)
10/2022 - 11/2022	1,552 (3.1%)	6,370 (10.4%)	7,922 (7.1%)	946 (4.5%)	3,302 (9.5%)	4,248 (7.6%)
Obesity						
0	33,381 (66.2%)	42,165 (69.0%)	75,546 (67.7%)	13,832 (65.3%)	23,895 (68.5%)	37,727 (67.3%)
1	17,017 (33.8%)	18,976 (31.0%)	35,993 (32.3%)	7,348 (34.7%)	11,005 (31.5%)	18,353 (32.7%)
PMCA*						
0	33,870 (67.2%)	40,976 (67.0%)	74,846 (67.1%)	13,482 (63.7%)	21,079 (60.4%)	34,561 (61.6%)
1	10,000 (19.8%)	11,189 (18.3%)	21,189 (19.0%)	4,382 (20.7%)	6,764 (19.4%)	11,146 (19.9%)
2	6,528 (13.0%)	8,976 (14.7%)	15,504 (13.9%)	3,316 (15.7%)	7,057 (20.2%)	10,373 (18.5%)
Negative tests prior entry						
0	2,337 (4.6%)	5,640 (9.2%)	7,977 (7.2%)	768 (3.6%)	2,966 (8.5%)	3,734 (6.7%)
1	34,077 (67.6%)	28,417 (46.5%)	62,494 (56.0%)	15,707 (74.2%)	17,303 (49.6%)	33,010 (58.9%)
2	10,514 (20.9%)	19,816 (32.4%)	30,330 (27.2%)	3,654 (17.3%)	11,012 (31.6%)	14,666 (26.2%)
>=3	3,470 (6.9%)	7,268 (11.9%)	10,738 (9.6%)	1,051 (5.0%)	3,619 (10.4%)	4,670 (8.3%)

*PMCA: Pediatric Medical Complexity Algorithm

Table 3. Estimated effectiveness of the BNT162b2 vaccine in preventing infection and severe diseases with SARS-CoV-2 in children and adolescents.

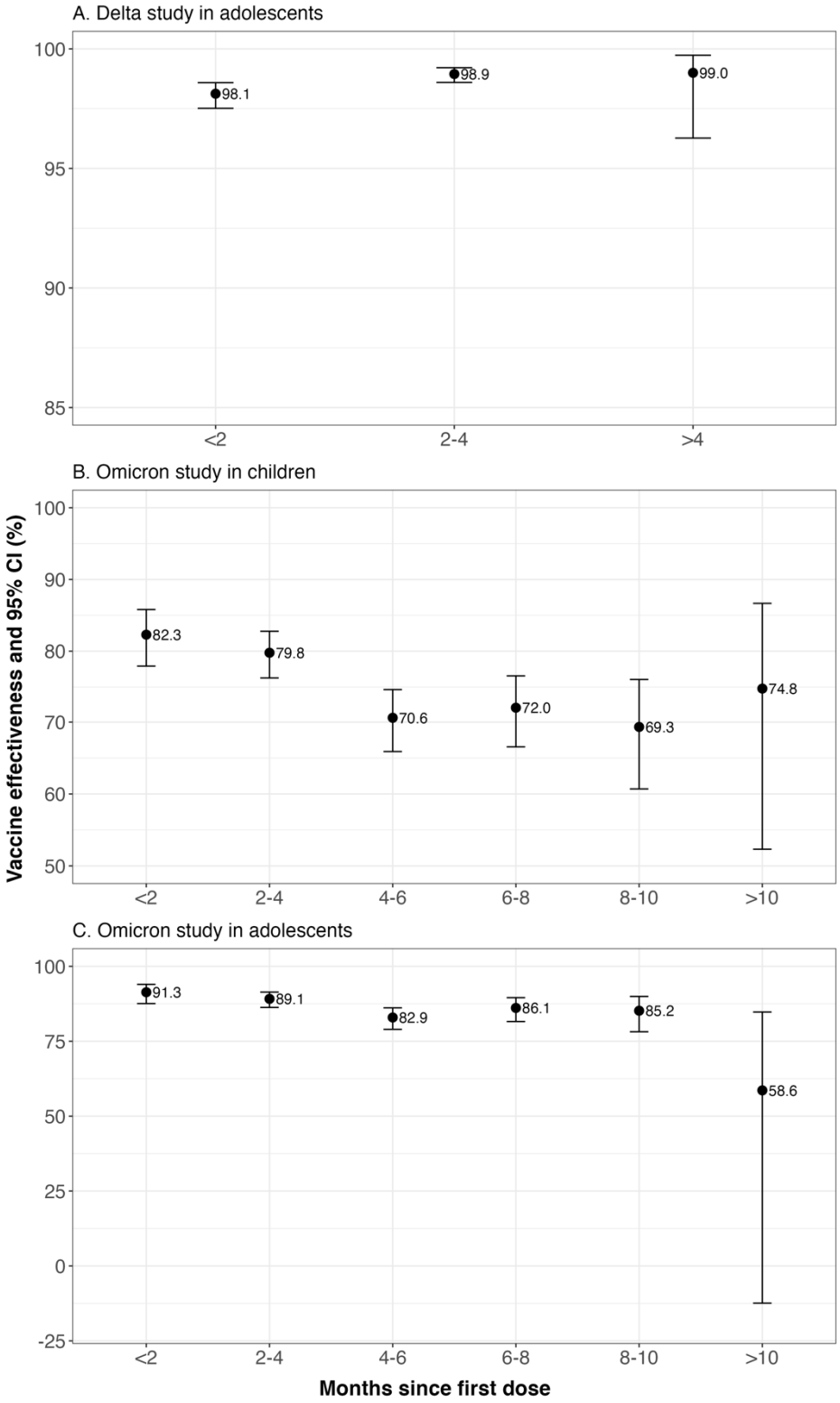
	Vaccinated	Unvaccinated	Overall	Vaccine effectiveness (in %) and 95% CI
		Delta study in adolescents		
Follow-up				
Total follow-up — no. of person-wk	644,162	398,906	1,043,068	
Median [Q1, Q3]	16 [12, 18]	13 [9, 17]	15 [10, 18]	
Absolute risk (in %)				
Documented infection	0.35	5.26	2.41	98.4 (98.1, 98.7)
Mild COVID-19	0.06	1.43	0.63	99.0 (98.5, 99.3)
Moderate or severe COVID-19	0.03	0.49	0.22	98.7 (97.4, 99.3)
ICU* admission with COVID-19	<0.01	0.05	< 0.03	99.0 (92.5, 99.9)
Cardiac complication	0.02	0.03	0.02	1.22 (0.34, 4.35)†
Age 12-15 yr				
Total follow-up — no. of person-wk	458,981	229,083	688,064	
Documented infection	0.34	5.52	2.28	99.0 (98.6, 99.3)
Age 16-21 yr				
Total follow-up — no. of person-wk	185,181	169,823	355,003	
Documented infection	0.36	4.91	2.64	97.0 (95.9, 97.8)
		Omicron study in children		
Follow-up				
Total follow-up — no. of person-wk	1,925,686	1,911,599	3,837,285	
Median [Q1, Q3]	44 [35, 46]	36 [19, 44]	40 [25, 45]	
Absolute risk (in %)				
Documented infection	1.89	5.46	3.85	74.3 (72.2, 76.2)
Mild COVID-19	0.54	1.55	1.09	73.5 (69.2, 77.1)
Moderate or severe COVID-19	0.19	0.67	0.45	75.5 (69.0, 81.0)
ICU admission with COVID-19	0.02	0.08	0.05	84.9 (64.8, 93.5)
Cardiac complication	<0.01	0.03	<0.02	0.28 (0.08, 0.95)†
Age 5-8 yr				
Total follow-up — no. of person-wk	1,101,418	1,254,819	2,356,236	
Documented infection	1.96	5.10	3.78	71.3 (68.2, 74.1)
Age 9-11 yr				
Total follow-up — no. of person-wk	824,268	656,780	1,481,049	
Documented infection	1.80	6.11	3.95	77.9 (75.1, 80.4)

Omicron study in adolescents				
Follow-up				
Total follow-up — no. of person-wk	772,176	1,113,561	1,885,736	
Median [Q1, Q3]	42 [30, 45]	37 [22, 44]	39 [25, 45]	
Absolute risk (in %)				
Documented infection	1.82	8.17	5.77	85.5 (83.8, 87.1)
Mild COVID-19	0.43	2.07	1.45	87.0 (83.5, 89.8)
Moderate or severe COVID-19	0.15	0.88	0.60	84.8 (77.3, 89.9)
ICU admission with COVID-19	<0.02	0.14	< 0.10	91.5 (69.5, 97.6)
Cardiac complication	<0.02	0.05	<0.04	0.10 (0.02, 0.57)†
Age 12-15 yr				
Total follow-up — no. of person-wk	524,053	654,315	1,178,368	
Documented infection	1.93	8.24	5.67	85.8 (83.6, 87.7)
Age 16-21 yr				
Total follow-up — no. of person-wk	248,123	459,246	707,368	
Documented infection	1.60	8.07	5.93	85.9 (82.7, 88.5)

*: ICU: Intensive Care Unit

†: relative risk in the vaccinated groups compared to the unvaccinated.

Figure 2. Stratified effectiveness of the BNT162b2 vaccine in preventing infection with SARS-CoV-2 in children and adolescents by 2-month intervals since receipt of the first dose



This is Exhibit F referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

MAJOR ARTICLE
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Severity of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Infection in Pregnancy in Ontario: A Matched Cohort Analysis

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Background. Pregnancy represents a physiological state associated with increased vulnerability to severe outcomes from infectious diseases, both for the pregnant person and developing infant. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic may have important health consequences for pregnant individuals, who may also be more reluctant than nonpregnant people to accept vaccination.

Methods. We sought to estimate the degree to which increased severity of SARS-CoV-2 outcomes can be attributed to pregnancy using a population-based SARS-CoV-2 case file from Ontario, Canada. Because of varying propensity to receive vaccination, and changes in dominant circulating viral strains over time, a time-matched cohort study was performed to evaluate the relative risk of severe illness in pregnant women with SARS-CoV-2 compared to other SARS-CoV-2 infected women of childbearing age (10–49 years old). Risk of severe SARS-CoV-2 outcomes was evaluated in pregnant women and time-matched nonpregnant controls using multivariable conditional logistic regression.

Results. Compared with the rest of the population, nonpregnant women of childbearing age had an elevated risk of infection (standardized morbidity ratio, 1.28), whereas risk of infection was reduced among pregnant women (standardized morbidity ratio, 0.43). After adjustment for confounding, pregnant women had a markedly elevated risk of hospitalization (adjusted odds ratio, 4.96; 95% confidence interval, 3.86–6.37) and intensive care unit admission (adjusted odds ratio, 6.58; 95% confidence interval, 3.29–13.18). The relative increase in hospitalization risk associated with pregnancy was greater in women without comorbidities than in those with comorbidities (*P* for heterogeneity, .004).

Conclusions. Given the safety of SARS-CoV-2 vaccines in pregnancy, risk-benefit calculus strongly favors SARS-CoV-2 vaccination in pregnant women.

Keywords. SARS coronavirus; pregnancy; COVID-19; epidemiology; respiratory disease; outcomes.

Pregnant individuals represent an important priority population for communicable disease prevention, for several reasons. The pregnant state results in changes in the immune system necessary for immune tolerance of the fetus [1, 2], and that may result in greater severity of some infections [1, 3–5]. Physiological changes associated with pregnancy, including metabolic and hormonal changes, and mechanical reduction in respiratory reserve, make pregnant individuals more vulnerable to respiratory impairment [5, 6]. Management of critical illness in pregnancy is challenging because of the distinct physiology of pregnant people and concerns around the use of some therapeutic agents, whereas critical illness in the pregnant individual may result in fetal demise [5, 7]. Finally, although vaccines in pregnancy, including those that prevent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, are safe and effective [8], their uptake may be limited because of aversion to pharmaceuticals on the part of both pregnant individuals and care providers [9, 10], as well as informational gaps due to the purposeful exclusion of pregnant individuals from clinical trials. Informed decisions around vaccine acceptance depend on accurate information on the risks of SARS-CoV-2 in the context of pregnancy.

SARS-CoV-2 has infected hundreds of millions of people since the disease emerged in 2019; it has also caused critical illness and death in millions [11]. In the United States, pregnant women have been found to be at a markedly elevated risk of critical illness from SARS-CoV-2 infection, as well as an elevated risk of stillbirth [12–14]. Initial analyses failed to identify an elevated risk of death among pregnant women with SARS-CoV-2 infection, but a subsequent reanalysis identified increased mortality risk, likely because of increased event numbers with the passage of time [13]. Although the per capita incidence of

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SARS-CoV-2-related illness and death has been lower in Canada than in the United States [15, 16], the pandemic has been the cause of tens of thousands of excess deaths in Canada, notwithstanding likely undercounting of SARS-CoV-2 attributable mortality [17]. A multiprovince Canadian surveillance group has reported elevated risk of hospitalization and critical illness associated with SARS-CoV-2 in pregnant individuals in Canada, but this report was published in early June 2021, just as the Delta variant of concern (VOC) was emerging, and relatively early in the Canadian SARS-CoV-2 vaccination effort [18, 19]. Furthermore, this report was largely descriptive, and did not adjust for confounding by VOC, vaccination, age, underlying comorbidity, or healthcare worker status.

The Canadian province of Ontario represents a large (population 14.6 million) and diverse jurisdiction, with high levels of SARS-CoV-2 vaccine coverage (approximately 80% as of December 2021) [20, 21]. Ontario has weathered multiple pandemic waves because of the original circulating variant of SARS-CoV-2, and latterly waves caused by the Alpha (spring 2021) and Delta (summer and autumn, 2021) VOC [22], with the Omicron VOC displacing Delta in December 2021 [23].

The province's rich data sources provide an opportunity for evaluation of the impact of SARS-CoV-2 in pregnant women, relative to other women of childbearing age, and with adjustment for important confounders. Our principal objective was to evaluate the relative morbidity associated with SARS-CoV-2 infection in pregnancy in Ontario. Secondary objectives were to evaluate the impact of infecting VOC and vaccination status on SARS-CoV-2 outcomes.

METHODS

Data Sources

As the likelihood of vaccination, the dominant SARS-CoV-2 variant, and the provincial public health response changed over time, we created a time-matched cohort of women infected with SARS-CoV-2 in pregnancy; nonpregnant controls were individuals with SARS-CoV-2 matched to pregnant women by date of positive laboratory test for SARS-CoV-2. Pregnant individuals and matched nonpregnant controls were identified in the Province's Case and Contact Management database as described elsewhere [24, 25]; we included only cases with a unique "pseudo-health card number," which permitted linkage with the provincial vaccination database. Control selection was limited to women of reproductive age. Because data were available by 10-year age bands, we considered "reproductive age" to encompass individuals aged 10 to 49 years. Data on comorbidities, healthcare worker status, and infecting variant were also available in the Case and Contact Management database. Comorbidities included neurologic disorders, asthma, renal conditions, blood disorders, cancer, cardiovascular conditions, liver conditions, and obesity. We included the period between 1 January 2020 and 4 January 2022 in the analysis.

Population denominators for the population overall and for women aged 10–49 years in Ontario, were obtained from Statistics Canada [20]. We estimated person-time at risk of SARS-CoV-2 infection among pregnant individuals based on reports of 107 855 live births and 482 stillbirths among women of childbearing age in Ontario between 1 January and 31 October 2021 [26]. Person-time at risk was adjusted based on an assumed duration of pregnancy of 40 weeks. To estimate person-time at risk of SARS-CoV-2 infection for nonpregnant women of childbearing age, we subtracted estimated annual person-time at risk among pregnant individuals from the population of women of childbearing age. The person-time at risk among those not classified as women of childbearing age was estimated as the total population size, minus the population of women of childbearing age. Variants were classified as non-variant of concern, N501Y+ variant (including the Alpha, Beta, and Gamma variants), or Delta variant, as described elsewhere [25]. Individuals were considered infected with the Omicron variant (B.1.1.529) if they had been identified as such through viral sequencing, if they were infected with a strain with S-gene target failure on polymerase chain reaction, or with the N501Y mutation, on or after 10 November 2021.

Vaccination information on cases and controls was extracted from the Province's COVaxON dataset [25], which includes dosage dates and vaccines used. To account for time to develop immunity, we considered individuals to have been vaccinated with a first dose of vaccine during time at risk 14 or more days after the date of their first vaccine dose; individuals were considered vaccinated with 2 doses of vaccine during time at risk 14 or more days after their second vaccine dose. A flow diagram outlining creation of the cohort is presented in Figure 1.

Analysis

We explored temporal trends in case incidence overall, in women aged 10–49 years, and in pregnant women, graphically and through calculation of standardized morbidity ratios (SMRs) as described previously [27]. Briefly, SMRs were estimated as incidence in pregnant women, or nonpregnant women of childbearing age, divided by incidence in the population overall. We calculated SMRs for the entire study period and by week. Confidence intervals for proportions were estimated based on standard errors for log-SMRs as previously [27].

We used multivariable conditional logistic regression models to estimate the risk of severe illness among our matched cohort while adjusting for age (treated as a 4-level ordinal variable), comorbidity, healthcare worker status, vaccination status, and infecting variant, all of which were selected a priori. Severe illness was defined as hospitalization or intensive care unit (ICU) admission. We were not able to include death as an outcome because fewer than 5 pregnant individuals died of SARS-CoV-2

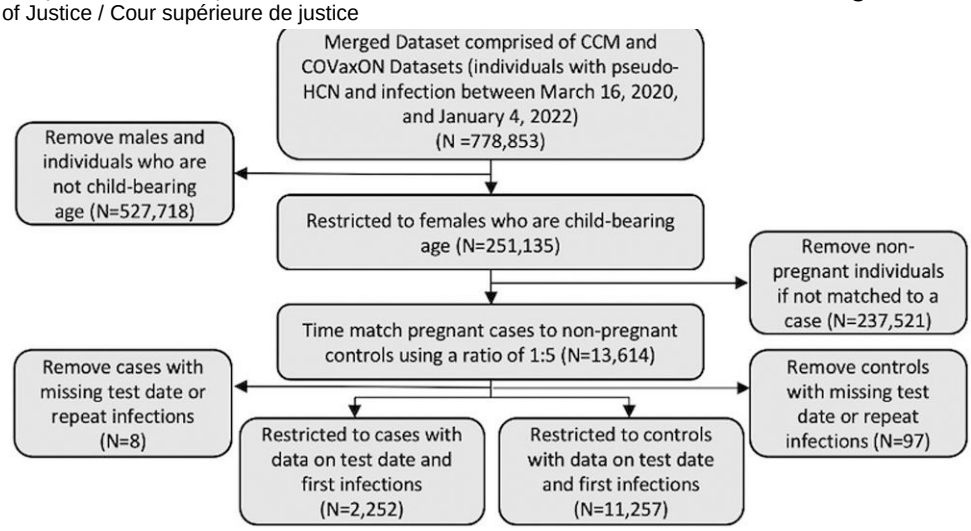

Figure 1. Flow diagram for creation of case-cohort sample. Abbreviations: CCM, Ontario's COVID-19 Contact Case Management Database; COVaxON, Ontario's COVID-19 vaccination database.

in Ontario at the time of analysis. Because of small numbers of adverse outcomes in individuals vaccinated with 3 doses, we treated vaccination as an ordinal variable with 3 levels (ie, unvaccinated, 1 dose, and 2 or more doses).

Because some pregnant individuals infected with SARS-CoV-2 might be hospitalized for monitoring, we performed an additional restriction analysis, in which risk of ICU was evaluated among pregnant and nonpregnant women admitted to hospital. We hypothesized that if pregnant individuals were admitted for precautionary reasons, they would be less likely to be admitted to ICU than nonpregnant controls, and if admission was due to severity of illness, pregnant individuals should be as or more likely than nonpregnant controls to be admitted to ICU. Because of lack of within-stratum variance in healthcare worker status and infecting variant among those admitted to hospital, we adjusted only for age, comorbidity, and vaccination in analyses restricted to hospitalized individuals.

We performed a series of exploratory restriction analyses within each stratum of comorbidity status, age group, vaccination status, or infecting variant variables to evaluate modification of the observed effects by these covariates in pregnant individuals and nonpregnant controls. Because conditional logistic regression models failed to converge for some of these models, we used logistic regression models with time modeled as a cubic term. Heterogeneity in adjusted odds ratios for hospitalization and ICU admission was evaluated using meta-analytic techniques (ie, graphically using forest plots, and statistically using the meta-analytic Q statistic). Our study was conducted in accordance with the STROBE guidelines for observational research [28], and received ethics approval from the Research Ethics Board at the University of Toronto.

RESULTS

Our final cohort consisted of 2252 pregnant women and 11 257 nonpregnant controls, with 5 controls for all but 3 cases (which had 4 matched controls each), with test dates between 16 March 2020, and 4 January 2022. Although the temporal pattern of infection risk in pregnant individuals and nonpregnant women of childbearing age mirrored risk in the population as a whole over time, risk was elevated in nonpregnant women of childbearing age (SMR 1.28) and decreased in pregnant women (SMR 0.43) (Figure 2).

In our matched cohort, pregnant individuals and nonpregnant controls differed significantly according to risk of hospitalization and ICU admission, as well as age distribution, vaccination status, healthcare worker status, and the presence of any significant comorbidity. Pregnant individuals were more likely than nonpregnant controls to have asthma, diabetes, or a diagnosed hematological disorder. There were no differences between pregnant women and nonpregnant controls with respect to infecting variant, likely because we created a time-matched cohort (Table 1).

Conditional logistic regression models for hospitalization and ICU admission are presented in Table 2. After adjusting for potential confounders, we identified a marked increase in risk of admission to hospital (adjusted odds ratio [aOR], 4.94; 95% confidence interval [CI], 3.85–6.34) and ICU admission (aOR, 6.58; 95% CI, 3.29–13.18) in pregnant individuals with SARS-CoV-2 infection compared with nonpregnant controls. We found no significant difference in risk of ICU admission between pregnant and nonpregnant women conditional on hospital admission after adjusting for comorbidity, age, vaccination status, and infecting variant (aOR, 1.30; 95% CI, .70–2.45).

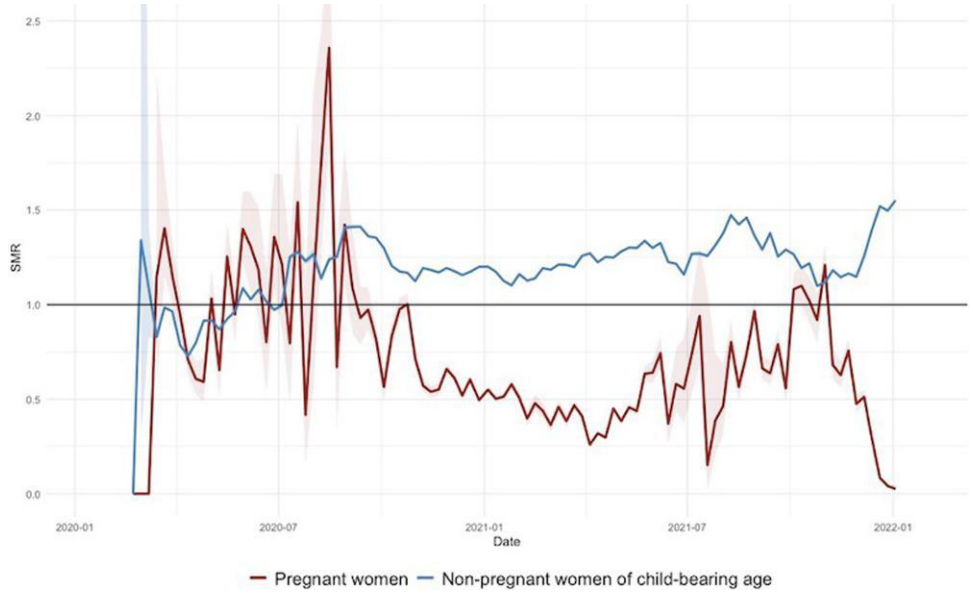

Figure 2. Standardized mortality ratios over time of SARS-CoV-2 cases in pregnant women and nonpregnant women of child-bearing age, Ontario, Canada, January 2020–January 2022. Line of reference at (black) depicts overall Ontario population. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SMR, standardized mortality ratio.

We performed unmatched logistic regression analyses, with adjustment for time trends and models restricted to individuals with similar comorbidity, vaccination or healthcare worker status, or age group. A summary of the heterogeneity in effect sizes for hospitalizations and ICU admission based on comorbidity status is displayed in Figure 3. The odds of hospitalization among pregnant individuals were significantly greater in analyses restricted to those without recorded comorbidities (aOR, 5.59; 95% CI, 4.34–7.20), compared with analyses restricted to those with comorbidities (aOR, 2.26; 95% CI, 1.28–3.99) (*P* for heterogeneity .029), but no heterogeneity in ICU admission risk by presence of comorbidity (Supplementary Table 1). We did not identify heterogeneity in the effect of pregnancy by infecting variant or vaccination status.

DISCUSSION

In a cohort of pregnant and nonpregnant women in Ontario, Canada, pregnancy was associated with a decreased risk of infection, but a markedly increased risk of severe illness following infection with SARS-CoV-2. We speculate that the decreased risk of infection in pregnant women may derive from greater adherence to public health guidance or avoidance of high-risk transmission settings. The large increase in risk of severe illness in pregnant women, conditional on infection, persisted after adjustment for age, comorbidity, vaccination status, and infecting variant. That pregnant and nonpregnant women were matched by approximate date of infection makes it unlikely that the large increase in hospitalization and ICU risk associated with pregnancy is due to varying propensity to receive vaccination over time or by temporal changes in circulating variants of concern. Furthermore, we did not see a diminished risk of ICU admission in hospitalized pregnant women when they were compared with other hospitalized women, suggesting that pregnant women were likely to have been admitted to hospital for severity of respiratory illness rather than simply for monitoring.

The excess risk associated with pregnancy was less pronounced when analyses were restricted to pregnant and nonpregnant women who had comorbidities, again suggesting that in otherwise healthy women, pregnancy itself is a factor that increases illness severity, whereas in women with comorbidities it becomes 1 of several factors that augments risk. The physiology of increased severity of respiratory virus infection in pregnancy is complex and not fully understood in the context of SARS-CoV-2. Although pregnant women have increased cardiac demand, diminished pulmonary reserve, and physiological impairment of the immune response (which serves to prevent rejection of the developing fetus), and these changes have been linked to worse outcome with pulmonary infection [5, 29], coronavirus disease 2019 (COVID-19) is not simply an infection of the respiratory system. Numerous vascular and hematological abnormalities occur following SARS-CoV-2 infection, and reports of elevated risk of stillbirth in infected individuals [12] and pathological changes reported in the placenta [30], may suggest that some of the excess morbidity we describe here is related to vascular and hematological disease rather than respiratory disease.

Table 1. Characteristics of Matched Study Cohort by Pregnancy Status, Ontario, Canada

	Pregnant Women	(%)	Nonpregnant Controls	(%)	Total	(%)	*P* value
N	2252	…	11 257	…	13 509	…	
Outcome	…	…	…	…	…	…	
Hospitalization	155	6.88	186	1.65	341	2.52	<.001
Intensive care unit admission	26	1.15	28	0.25	54	0.40	<.001
Age group, y	…	…	…	…	…	…	<.001
10–19	33	1.47	1876	16.67	1909	14.13	
20–29	921	40.90	3453	30.67	4374	32.38	
30–39	1208	53.64	3006	26.70	4214	31.19	
40–49	90	4.00	2922	25.96	3012	22.30	
Vaccination status	…	…	…	…	…	…	.004
Unvaccinated	2009	89.21	9756	86.67	11 765	87.09	
Single dose	53	2.35	342	3.04	395	2.92	
≥2 doses	190	8.44	1159	10.30	1313	9.72	
Healthcare worker	295	13.10	1018	9.04	1313	9.72	<.001
Any significant comorbidity	230	10.21	749	6.65	979	7.25	<.001
Asthma	94	4.17	301	2.67	395	2.92	<.001
Hematological disease	46	2.04	121	1.07	167	1.24	<.001
Cardiac disease	21	0.93	114	1.01	135	1.00	.727
Diabetes	57	2.53	123	1.09	180	1.33	<.001
Renal disease	7	0.31	23	0.20	30	0.22	.327
Neurological disease	9	0.40	35	0.31	44	0.33	.500
Obesity	11	0.49	42	0.37	53	0.39	.424
Immune compromised	9	0.40	81	0.72	90	0.67	.088
Infecting variant	…	…	…	…	…	…	.149
Delta variant of concern	345	15.32	1609	14.29	1954	14.46	
N501Y+ variant of concern	433	19.23	2141	19.02	2574	19.05	
Nonvariant of concern	103	4.57	530	4.71	633	4.69	
Unknown variant of concern	1321	58.66	6680	59.34	8001	59.23	
Other variant of concern	25	1.11	96	0.85	121	0.90	
Omicron variant of concern	25	1.11	201	1.79	226	1.67	

[a]Absolute numbers not shown for deaths, or specific comorbidities (liver disease and chronic obstructive pulmonary disease) from concern with identifiability with small cell sizes (N < 5). Differences between pregnant and nonpregnant women were nonsignificant ($P > .50$) for all of these outcomes and characteristics. Proportions compared using χ^2 tests.

Although our primary aim in this study was to evaluate the impact of pregnancy on risk of severe illness with SARS-CoV-2, vaccination, including partial vaccination, was associated with a marked reduction in hospital admission risk and ICU admission in multivariable analyses, notwithstanding that in this case-only analysis, all vaccinated women had, by definition, experienced breakthrough infections. Again, given the safety of these vaccines in pregnancy [8], and the markedly elevated risk of severe illness in pregnancy, risk calculus strongly favors vaccination for pregnant women.

Like any observational study, ours is subject to several limitations. The recent emergence of the Omicron variant makes us unable to explore the relative virulence of this variant in pregnancy in the current paper [23]. Our estimates may also be subject to residual confounding by incompletely ascertained factors, including presence of underlying medical conditions. Comorbidity data were not validated; comorbidities were classified as "present" by the local public health personnel evaluating the case, and as such may have been misclassified in some cases. However, the very large effect size associated with pregnancy means that the magnitude of effect of putative confounding by unmeasured factors needed to explain away these associations would be implausibly large [31]. Furthermore, for pregnancy estimates to be inflated by unmeasured confounding by comorbidity, differential underreporting of comorbidities in pregnant cases would need to have occurred; we would expect that, if anything, pregnancy status would result in more complete ascertainment of medical historical factors. This is consistent with the fact that comorbidities were more commonly reported in pregnant women in our cohort. Because achieving pregnancy may be challenging in individuals with multiple comorbidities, a higher prevalence of comorbidities in pregnant individuals is contrary to expectations and likely suggests increased ascertainment in these individuals.

In summary, we identify a large increase in risk of hospitalization and ICU admission in pregnant women infected with SARS-CoV-2 virus, relative to female controls of childbearing age. This effect was not explained by comorbidity or vaccination status, and indeed, the relative increase in risk with pregnancy was greater when we restricted our analyses to women

Table 2. Results of Multivariable Conditional Logistic Regression Model on Hospitalization and ICU Admission in Pregnant Women Because of SARS-CoV-2 Infection

Covariate	Crude Odds Ratio	LCL	UCL	*P* value	Adjusted Odds Ratio	LCL	UCL	*P* value
				Hospitalization				
Pregnancy	4.46	3.57	5.56	<.001	4.94	3.85	6.34	<.001
Vaccination	…	…	…		…	…	…	…
Unvaccinated (Referent)	1.00	…	…		1.00	…	…	…
1 dose	0.45	0.20	1.01	.054	0.51	0.21	1.24	.137
≥2 doses	0.08	0.03	0.22	<.001	0.07	0.02	0.23	<.001
Age group (per 10-y increase)	1.44	1.27	1.64	<.001	1.69	1.45	1.98	<.001
Healthcare worker	0.42	0.25	0.71	.001	0.34	0.20	0.58	<.001
Comorbidity	3.65	2.58	5.18	<.001	3.59	2.39	5.38	<.001
Infecting variant[a]	…	…	…		…	…	…	…
Delta VOC	1.04	0.62	1.76	.882	0.86	0.47	1.59	.638
N501Y+ VOC	0.90	0.58	1.40	.646	0.82	0.50	1.34	.430
				ICU Admission				
Pregnancy	4.64	2.72	7.92	<.001	6.58	3.29	13.18	<.001
Vaccination	…	…	…		…	…	…	…
Unvaccinated (referent)	1.00	…	…		1.00	…	…	…
1 dose	0.33	0.04	2.62	.295	0.34	0.02	4.87	.424
≥2 doses	0.25	0.03	2.04	.197	0.25	0.02	2.55	.241
Age group	1.53	1.13	2.08	.006	1.71	1.11	2.64	.015
Healthcare worker	0.16	0.03	0.78	.024	0.09	0.02	0.47	.005
Comorbidity	7.91	3.33	18.83	<.001	8.71	2.88	26.36	<.001
Infecting variant[a]	…	…	…		…	…	…	…
Delta VOC	5.06	0.80	32.10	.085	13.50	0.53	343.19	.115
N501Y+ VOC	1.42	0.48	4.20	.522	0.94	0.25	3.45	.920

Abbreviations: ICU, intensive care unit; LCL, 95% lower confidence limit; UCL, 95% upper confidence limit; VOC, variant of concern.

[a]Infection with strains classified as non-VOC, other VOC status (including Omicron VOC), or VOC status unknown used as referent.

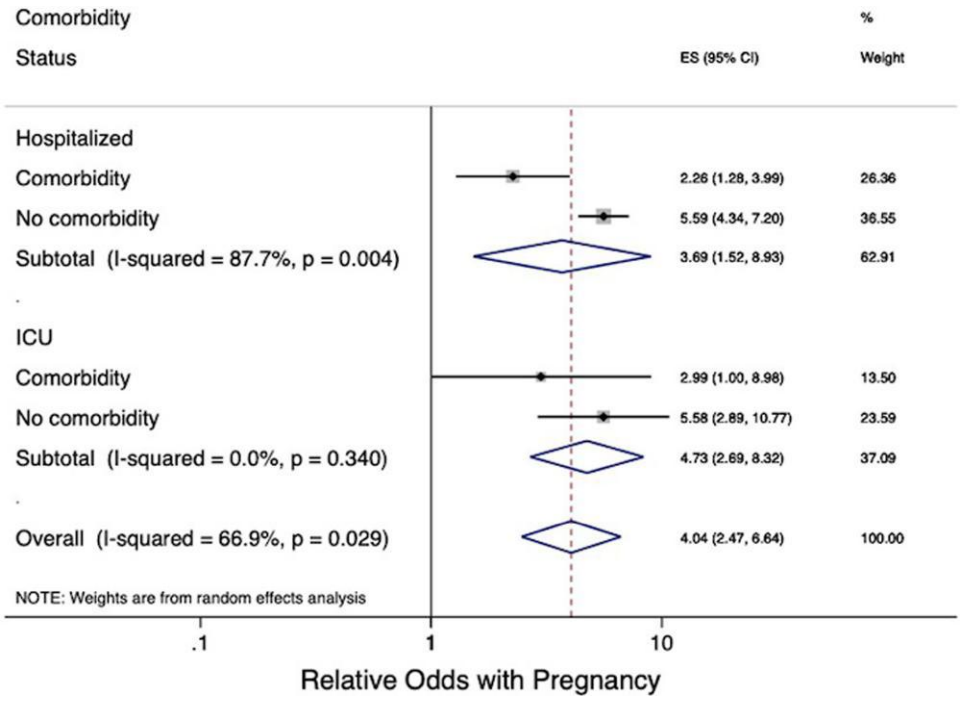

Figure 3. Forest plot summarizing heterogeneity in effect sizes between comorbidity status and severe outcome. Results are stratified on outcome (hospitalization and intensive care unit admission).

without medical comorbidities. Vaccination markedly reduced hospitalization and ICU admission risk in all women, pregnant and nonpregnant, in this study, and should be strongly encouraged in pregnancy.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank the staff at Public Health Ontario and Ontario's public health units for collecting, sequencing, analyzing, and providing access to the data used for this analysis.

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Potential conflicts of interest. D. N. F. has served on advisory boards related to influenza and SARS-CoV-2 vaccines for Seqirus, Pfizer, Astrazeneca, and Sanofi-Pasteur Vaccines, and has served as a legal expert on issues related to COVID-19 epidemiology for the Elementary Teachers Federation of Ontario and the Registered Nurses Association of Ontario. A. R. T. was employed by the Public Health Agency of Canada when this research was conducted. The work does not represent the views of the Public Health Agency of Canada. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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This is Exhibit G referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

MAJOR ARTICLE
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Relative Virulence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Among Vaccinated and Unvaccinated Individuals Hospitalized With SARS-CoV-2

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Background. The rapid development of safe and effective vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a singular scientific achievement. Confounding due to health-seeking behaviors, circulating variants, and differential testing by vaccination status may bias analyses toward an apparent increase in infection severity following vaccination.

Methods. We used data from the Ontario, Canada, Case and Contact Management Database and a provincial vaccination dataset (COVaxON) to create a time-matched cohort of individuals who were hospitalized with SARS-CoV-2 infection. Vaccinated individuals were matched to up to 5 unvaccinated individuals based on test date. Risk of intensive care unit (ICU) admission and death were evaluated using conditional logistic regression.

Results. In 20 064 individuals (3353 vaccinated and 16 711 unvaccinated) hospitalized with infection due to SARS-CoV-2 between 1 January 2021 and 5 January 2022, vaccination with 1, 2, or 3 doses significantly reduced the risk of ICU admission and death. An inverse dose–response relationship was observed between vaccine doses received and both outcomes (adjusted odds ratio [aOR] per additional dose for ICU admission, 0.66; 95% confidence interval [CI], .62 to .71; aOR for death, 0.78; 95% CI, .72 to .84).

Conclusions. We identified decreased virulence of SARS-CoV-2 infections in vaccinated individuals, even when vaccines failed to prevent infection sufficiently severe to cause hospitalization. Even with diminished efficacy of vaccines against infection with novel variants of concern, vaccines remain an important tool for reduction of ICU admission and mortality.

Keywords. epidemiology; SARS-CoV-2; vaccination; pandemic; outcomes.

The global pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has sickened hundreds of millions of people and killed millions [1]. The rapid development of safe and effective vaccines against the virus has been a singular scientific achievement and has likely prevented many more illnesses and deaths [2–4]. However, the ongoing emergence of novel viral variants remains a challenge, with reduced protection against infection seen with the B.1.529 (Omicron) variant that emerged in fall 2021 [5]; though vaccines seem to continue to prevent severe illness, resulting in reduced hospitalizations, intensive care unit (ICU) admissions, and deaths. Indeed, the effectiveness of vaccination for prevention of severe outcomes reflects 2 effects that may be difficult to disentangle: prevention of infection and prevention of severe outcomes even when vaccination does not prevent infection. Studies may be further complicated by factors such as decreased propensity to test vaccinated individuals with mild symptoms of respiratory illness, which could produce biases that result in increased apparent severity of infection following vaccination.

Previously, we evaluated the effectiveness of SARS-CoV-2 vaccines to prevent infection in the Canadian province of Ontario [6]. However, vaccines that result in attenuation of severity, even among individuals in whom they failed to protect against infection, would be of considerable value to vaccinated individuals and to the wider community by limiting consumption of scarce critical care resources. We sought to study the impact of prior vaccination on severity of illness among individuals admitted to the hospital with SARS-CoV-2 in Ontario, Canada, because a study limited to hospitalized individuals should limit biases introduced by differential testing according to disease severity and vaccination status. As both the propensity to receive vaccination and the dominant viral variant of concern (VOC) has varied over the course of the pandemic in Ontario, we used a time-matched cohort to evaluate the adjusted risk of ICU admission and death in vaccinated and unvaccinated

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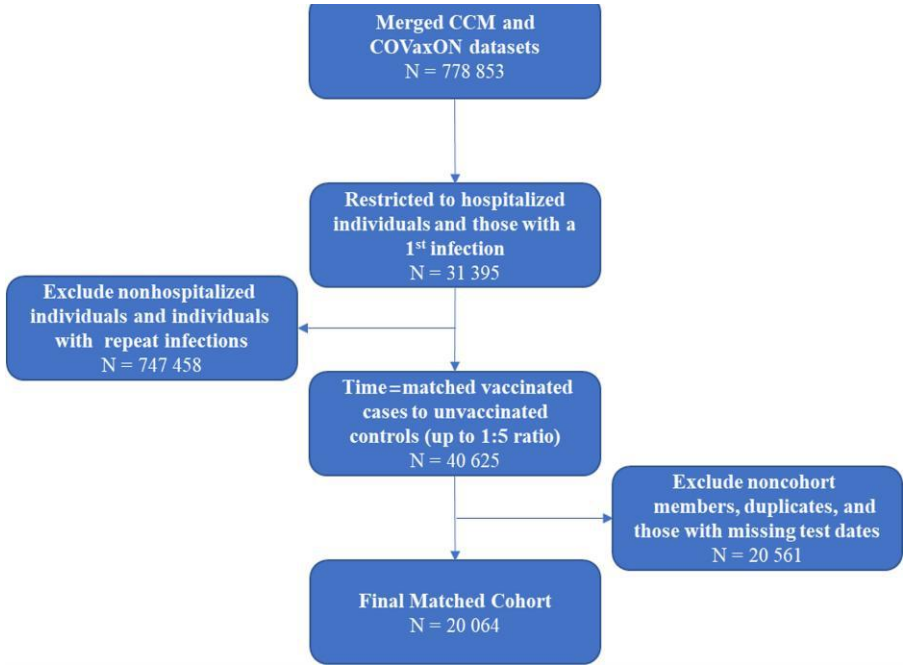

Figure 1. Flow diagram for the creation of the matched cohort. Abbreviations: CCM, Case and Contact Management System (Ontario's line list database); COVaxON, Ontario provincial vaccination database.

individuals with identically timed infection admitted to Ontario hospitals. Our primary objective was to determine whether the risks of ICU admission and death were diminished significantly by vaccination among individuals whose vaccination failed to prevent hospitalization. We also performed exploratory analyses to determine whether protective effects were modified by an individual's characteristics or by infecting viral variant.

METHODS

Data Sources

We created a time-matched cohort of individuals who were hospitalized due to SARS-CoV-2 infection between 1 January 2021 and 5 January 2022. Individuals who had received 1, 2, or 3 doses of a SARS-CoV-2 vaccine were included as exposed and were matched to unvaccinated individuals based on the test date for SARS-CoV-2. Each vaccinated individual was matched with up to 5 unvaccinated individuals who acted as controls [7]. We identified vaccinated and unvaccinated SARS-CoV-2 cases in the province's Case and Contact Management (CCM) System, as described elsewhere [8, 9].

We included only cases with a unique "pseudo-health card number," permitting linkage with the provincial vaccination COVaxON database [9], which provided information on dose administration, dates, and vaccines used. We considered individuals to be vaccinated 14 days or more after vaccine dose administration [10]. We used the date of positive testing as a surrogate for onset of infection; when individuals had a test date <14 days from a dose of vaccine, they were considered to be not protected by that dose. For example, an individual tested for infection 2 days after their second dose of vaccine would be considered single-dose vaccinated. While information on third vaccine doses was available in COVaxON, third doses were not widely available at the time of the analysis, and a small number of hospitalized individuals had received a third dose of vaccine. Consequently, we evaluated individuals based on the number of vaccine doses received and by classifying them as unvaccinated, single-dose vaccinated, double-dose vaccinated, or triple-dose vaccinated.

There have been several waves of the SARS-CoV-2 pandemic in Ontario, with different infecting variants prevalent for each. Initial waves were driven by the Wuhan variant, then waves were driven by Alpha in spring 2021, Delta in summer and fall 2021 [9], and finally Omicron in December 2021 [11]. As such, infecting viral variants in our analysis were classified as non-VOC, N501Y+ variant (including the Alpha, Beta, and Gamma variants) or Delta variant, as described elsewhere [9]. Individuals were considered infected with the Omicron variant (B.1.1.529) if they had been identified as such through whole-genome sequencing or if they were infected on or after 10 November 2021 with a strain with S-gene target failure or the N501Y mutation. Individuals were also considered infected with the Omicron variant if they had been classed as infected with an unknown VOC after 13 December 2022. A flow diagram outlining creation of the cohort is presented in Figure 1.

Analysis

We used our matched cohort to calculate the risk of ICU admission and death among those hospitalized due to SARS-CoV-2 using conditional logistic regression models. The models were specified a priori to adjust for age category (treated as a 9-level ordinal variable), sex, healthcare worker status, long-term care residence, comorbidity, and infecting variant. Vaccine status was treated as a 4-level nominal variable (0, 1, 2, or 3 doses) where 0 doses was used as the referent. Due to the rarity of deaths in healthcare workers, healthcare worker status was not included in models used to evaluate risk of death.

We also performed exploratory restriction analyses in which vaccinated and unvaccinated cases were limited to a single infecting variant (non-VOC, N501Y+ VOC, Delta VOC, or Omicron VOC) and additional exploratory analyses were conducted in which unvaccinated individuals were compared with individuals vaccinated exclusively with viral vector vaccines or to individuals vaccinated exclusively with messenger RNA (mRNA) vaccines. As conditional logistic regression models failed to converge for some of these models, we modeled the effect of vaccination using unmatched logistic regression models, with time trend modeled as a cubic trend function. We investigated heterogeneity in the adjusted odds ratios (aORs) for ICU admission and death according to infecting variant or vaccine product used using meta-analytic techniques (ie, graphically using forest plots, statistically using the meta-analytic Q statistic, and through construction of meta-regression models). The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology Guidelines for observational research [12] and received ethics approval from the University of Toronto Research Ethics Board.

Table 1. Baseline Characteristics of the Matched Cohort

Characteristic	Vaccinated	(%)	Unvaccinated Controls	(%)	Total	(%)	*P* Value
N	3353		16 711		20 064		
Outcome							
Intensive care unit admission	467	13.93	3819	22.85	4286	21.36	<.001
Death	517	15.42	1849	11.06	2366	11.79	<.001
Age group, y							
0–9 or 10–19[a]	32	0.95	1038	6.21	1070	5.33	<.001
20–29	52	1.55	905	5.42	957	4.77	
30–39	96	2.86	1665	9.96	1761	8.78	
40–49	142	4.24	2195	13.14	2337	11.65	
50–59	281	8.38	3108	18.60	3389	16.89	
60–69	576	17.18	3376	20.20	3952	19.70	
70–79	798	23.80	2669	15.97	3467	17.28	
80+	1376	41.04	1754	10.50	3130	15.60	
Male	1838	54.82	8935	53.47	10 773	53.69	.135
Healthcare worker	16	0.48	88	0.53	104	0.52	.716
Long-term care	102	3.04	93	0.56	195	0.97	<.001
Comorbidity							
Any significant comorbidity	1088	32.45	3242	19.40	4330	21.58	<.001
Asthma	79	2.36	344	2.06	423	2.11	.274
Hematological disease	47	1.40	136	0.81	183	0.91	.001
Cardiac disease	578	17.24	1662	9.95	2240	11.16	<.001
Chronic obstructive pulmonary disease	142	4.24	276	1.65	418	2.08	.628
Diabetes	344	10.26	1110	6.64	1454	7.25	<.001
Renal disease	138	4.12	226	1.35	364	1.81	<.001
Neurological disease	90	2.68	186	1.11	276	1.38	<.001
Obesity	49	1.46	244	1.46	293	1.46	.996
Liver disease	35	1.04	73	0.44	108	0.54	<.001
Immune compromise	215	6.41	448	2.68	663	3.30	<.001
Infecting variant of concern							
Not detected	69	2.06	432	2.59	501	2.50	<.001
N501Y+	1056	31.49	5648	33.80	6704	33.41	
Delta	954	28.45	5362	32.09	6316	31.48	
Presumptive Omicron	645	19.24	2917	17.46	404	2.01	
Other	49	1.46	207	1.24	256	1.28	
Unknown	580	17.30	2145	12.84	5883	29.32	

[a]Age groups combined due to small cells (≤5).

RESULTS

The final matched cohort consisted of 3353 vaccinated individuals and 16 711 unvaccinated individuals. Most cohort members were aged ≥50 years (69.47%), and most were infected with the N501Y+ VOC (33.41%). The majority of the cohort were male (53.69%), and 21.58% of individuals had a recorded major medical comorbidity. In univariable analyses, vaccinated and unvaccinated individuals differed significantly according to age group, residence in long-term care, comorbidity status, and infecting variant (Table 1). Among vaccinated individuals, 51.12% had received 1 dose, 44.23% had received 2 doses, and only 4.65% had received 3 doses.

We fit 2 conditional logistic regression models to assess the risk of being admitted to the ICU and the risk of death in hospitalized individuals (Table 2). Compared with no vaccination, vaccination with a single dose of vaccine (aOR, 0.57; 95% confidence interval [CI], .49 to .66), with 2 doses (aOR, 0.51; 95% CI, .44 to .60), or with 3 doses (aOR, 0.56; 95% CI, .34 to .93) significantly reduced ICU admission risk. When vaccination status was treated as a 4-level ordinal variable, we identified a significant inverse dose–response relationship between vaccine doses received and ICU admission risk (aOR per additional dose, 0.66; 95% CI, .62 to .71).

Vaccination also significantly decreased the risk of death conditional on hospitalization for SARS-CoV-2 (aOR for a single vaccine dose, 0.5; 95% CI, .44 to .59; aOR for 2 doses, 0.48; 95% CI, .40 to .56; and aOR for 3 doses, 0.52; 95% CI, .31 to .86). We identified a significant inverse dose–response relationship between the number of vaccine doses received and risk of death (aOR per additional dose, 0.78; 95% CI, .72 to .84).

We also performed an exploratory subgroup analysis on the odds of ICU admission and death stratified by the infecting variant. Heterogeneity in the aORs for ICU admission and death is shown in Figure 2. There was no significant heterogeneity between variants within each outcome. However, there was significant heterogeneity in vaccine effects based on the outcome used, with greater protection against ICU admission

Table 2. Odds Ratios and 95% Confidence Intervals from Conditional Logistic Regression on Intensive Care Unit Admission and Death Due to Severe Acute Respiratory Syndrome Coronavirus 2

Covariate	Crude OR	Lower CI	Upper CI	*P* Value	Adjusted OR[a]	Lower CI	Upper CI	*P* Value
Intensive care unit admission								
Vaccinations received								
Unvaccinated (referent)	1.00				1.00			
1 dose	0.57	.49	.66	<.001	0.51	.44	.59	<.001
2 doses	0.51	.44	.60	<.001	0.48	.40	.56	<.001
3 doses	0.56	.34	.93	.024	0.52	.31	.86	.011
Age group (per 10-year increase)	1.03	1.01	1.05	.001	1.07	1.05	1.09	<.001
Male	1.42	1.32	1.53	<.001	1.43	1.33	1.55	<.001
Healthcare worker	0.67	.38	1.18	.164	0.76	.43	1.35	.348
Long-term care	0.39	.23	.69	.001	0.43	.25	.75	.003
Any significant comorbidity	1.08	.98	1.18	.104	1.12	1.02	1.22	.019
Infecting VOC								
Non-VOC, unknown Other (referent)	1.00				1.00			
N501Y+	1.01	.89	1.15	.831	1.16	1.01	1.33	.040
Delta	1.80	1.61	2.01	<.001	1.78	1.56	2.04	<.001
Presumptive Omicron	0.50	.39	.65	<.001	1.04	.77	1.39	.816
Death								
Vaccinations received								
Unvaccinated (Referent)	1.00				1.00			
1 dose	1.52	1.32	1.75	<.001	0.68	.58	.80	<.001
2 doses	1.38	1.17	1.63	<.001	0.62	.51	.74	<.001
3 doses	1.98	1.05	3.73	.034	0.83	.42	1.61	.575
Age group (per 10-year increase)	1.69	1.63	1.75	<.001	1.77	1.70	1.84	<.001
Male	1.55	1.41	1.71	<.001	1.75	1.58	1.95	<.001
Long-term care	3.39	2.22	5.17	<.001	1.67	1.05	2.66	.029
Any significant comorbidity	1.66	1.50	1.85	<.001	1.40	1.25	1.57	<.001
Infecting VOC								
Non-VOC, unknown Other (referent)	1.00				1.00			
N501Y+	1.09	.93	1.27	.301	1.06	.89	1.27	.517
Delta	1.33	1.15	1.54	<.001	1.47	1.22	1.77	<.001
Presumptive Omicron	1.31	.89	1.93	.172	2.71	1.69	4.33	<.001

Abbreviations: CI, confidence interval; OR, odds ratio; VOC, variant of concern.

[a]Intensive care unit admission adjusted for age group, sex, healthcare worker, long-term care, comorbidity, and infecting variant. Death adjusted for age group, sex, long-term care, comorbidity, and infecting variant.

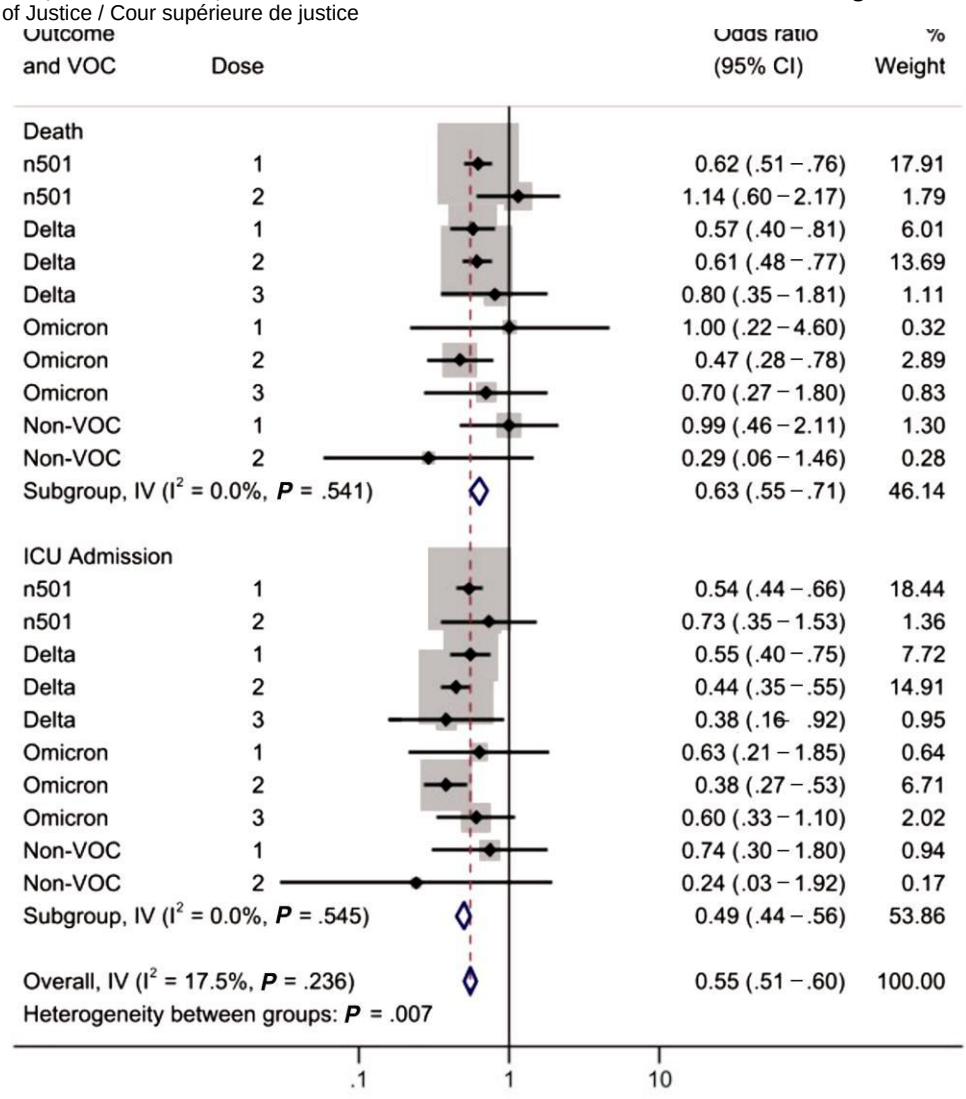

Figure 2. Forest plot to evaluate heterogeneity between estimates by infecting variant and outcome. The analysis is stratified by outcome, with results for death in the upper rows and ICU admission below. Abbreviations: CI, confidence interval; ICU, intensive care unit; IV, contribution of within-stratum variance to overall variance; n501, N501Y-positive variant; VOC, variant of concern.

than death ($P < .05$). When we created a meta-regression model that included vaccine type (mRNA vs viral vector vaccine), dose number, and outcome (death vs ICU), we found that vaccination was more protective against ICU admission than against death (27% relative reduction in risk; 95% CI, 12% to 39%), and mRNA vaccines were more protective against ICU admission and death than viral vector vaccines (36% relative reduction in risk; 95% CI, 10% to 55%). Results of stratified models and meta-regression are presented in detail in Supplementary Tables 1–3.

DISCUSSION

In a cohort of vaccinated and unvaccinated individuals matched on infection timing and hospitalization with SARS-CoV-2 infection in Ontario, Canada, vaccination was associated with a decreased risk of ICU admission and death after adjustment for confounding factors such as age, sex, health-care worker status, long-term care residence, comorbidity status, and infecting variant. A reduced risk of severe outcomes with increased number of vaccine doses received was also seen. The time-matched nature of our design suggests that our findings are unlikely to be due to changes in vaccine prioritization or dominant circulating variants over time. Restriction to hospitalized individuals makes it less likely that our findings are biased by differential testing in vaccinated and unvaccinated individuals. We postulate that this might result in confounding and selection bias, inasmuch as vaccinated individuals may be less likely to be tested for mild symptoms of infection but may be more likely to be tested overall.

The significant protective effects of vaccination against ICU admission and death were seen for both the N501Y+ and Delta variants in exploratory restriction analyses. While we did not identify significant protection against the Omicron variant in restriction analyses, this likely reflects low statistical power due to recent Omicron emergence. We found no evidence for heterogeneity in effect according to infecting variant. We did, however, identify greater protection against ICU admission than against death with vaccines. It is possible that this relates to residual confounding by long term care residence status, which independently reduced the likelihood of ICU admission while increasing the risk of death.

Protection against infection and symptomatic infection has declined with the emergence of novel VOCs, most notably the Omicron variant [5, 13–16]. While recent data suggest that booster doses of mRNA vaccines substantially restore vaccine efficacy [15, 16], our analysis shows that prior partial vaccination can provide benefits to individuals and health systems even when vaccines fail to prevent infection, or even hospitalization, and remains an important pillar of the public health response to the SARS-CoV-2 pandemic.

Our approach to this analysis emphasized adjustment for confounding both in the design (via matching) and analysis (via multivariable regression). The importance of adjustment for confounding when evaluating vaccine efficacy against SARS-CoV-2 relates to the differential risk profiles of vaccinated and unvaccinated individuals. For example, in Ontario, older individuals and those with medical comorbidities who are expected to have worse outcomes if infected with SARS-CoV-2 have been prioritized for vaccination. This so-called confounding by indication is likely to diminish the apparent crude efficacy of vaccines, as is seen in our unadjusted analyses, simply because those who are more likely to be vaccinated are also more likely to experience adverse outcomes of infection, independent of vaccination status. While confounding by age and comorbidity might be expected, we also adjusted by other factors, including biological sex. As in this analysis, we previously identified male sex as an independent risk factor for poor outcomes in individuals with SARS-CoV-2 infection [17, 18].

The effects we demonstrate here are not unique to SARS-CoV-2, and we have previously identified similar effects with prior pneumococcal and influenza vaccination [19, 20]. As with that earlier work, an important limitation here is the inability to ensure that the effects we observe are not at least in part due to residual confounding. This is a potential limitation of any cohort study and is one that will be the focus of future work. The relatively recent emergence of the Omicron variant and the lags associated with critical illness and death result in the lack of statistical power to estimate Omicron-specific protections, as noted above. Unfortunately, ongoing high rates of SARS-CoV-2 hospitalizations, critical illnesses, and deaths in Ontario mean that we will be able to address this potential limitation in the months ahead.

In summary, we identified a decrease in the risk of ICU admission and death in hospitalized, vaccinated individuals compared with hospitalized, unvaccinated individuals, matched for infection timing, in Ontario, Canada. Our analysis further emphasizes the critical importance of high rates of vaccination for protection of community health and reduction of the impacts of SARS-CoV-2 on ICU capacity during the pandemic.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. D. N. F. has served on advisory boards related to influenza and severe acute respiratory syndrome coronavirus 2 vaccines for Seqirus, Pfizer, AstraZeneca, and Sanofi-Pasteur Vaccines and has served as a legal expert on issues related to coronavirus disease 2019 epidemiology for the Elementary Teachers Federation of Ontario and the Registered Nurses Association of Ontario. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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This is Exhibit H referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

PLOS ONE

RESEARCH ARTICLE

Vaccine effectiveness against hospitalization among adolescent and pediatric SARS-CoV-2 cases between May 2021 and January 2022 in Ontario, Canada: A retrospective cohort study

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Abstract

Background

Vaccines against SARS-CoV-2 have been shown to reduce risk of infection as well as severe disease among those with breakthrough infection in adults. The latter effect is particularly important as immune evasion by Omicron variants appears to have made vaccines less effective at preventing infection. Therefore, we aimed to quantify the protection conferred by mRNA vaccination against hospitalization due to SARS-CoV-2 in adolescent and pediatric populations.

Methods

We retrospectively created a cohort of reported SARS-CoV-2 case records from Ontario's Public Health Case and Contact Management Solution among those aged 4 to 17 linked to vaccination records from the COVaxON database on January 19, 2022. We used multivariable logistic regression to estimate the association between vaccination and hospitalization among SARS-CoV-2 cases prior to and during the emergence of Omicron.

Results

We included 62 hospitalized and 27,674 non-hospitalized SARS-CoV-2 cases, with disease onset from May 28, 2021 to December 4, 2021 (Pre-Omicron) and from December 23, 2021 to January 9, 2022 (Omicron). Among adolescents, two mRNA vaccine doses were associated with an 85% (aOR = 0.15; 95% CI: [0.04, 0.53]; p<0.01) lower likelihood of hospitalization among SARS-CoV-2 cases caused by Omicron. Among children, one mRNA vaccine dose was associated with a 79% (aOR = 0.21; 95% CI: [0.03, 0.77]; p<0.05) lower likelihood of hospitalization among SARS-CoV-2 cases caused by Omicron. The calculation of E-values, which quantifies how strong an unmeasured confounder would need to be to nullify our findings, suggest that these effects are unlikely to be explained by unmeasured confounding.

170360, https://cihr-irsc.gc.ca/e/193.html). The doctoral training of AES is supported by the University of Toronto Connaught International Doctoral Scholarship (https://connaught.research.utoronto.ca). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: DNF has served on advisory boards related to influenza and SARS-CoV-2 vaccines for Seqirus, Pfizer, AstraZeneca, and Sanofi-Pasteur Vaccines, and has served as a legal expert on issues related to COVID-19 epidemiology for the Elementary Teachers Federation of Ontario and the Registered Nurses Association of Ontario. ART was employed by the Public Health Agency of Canada when the research was conducted. The work does not represent the views of the Public Health Agency of Canada. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

Conclusions

Despite immune evasion by SARS-CoV-2 variants, vaccination continues to be associated with a lower likelihood of hospitalization among adolescent and pediatric Omicron (B.1.1.529) SARS-CoV-2 cases, even when the vaccines do not prevent infection. Continued efforts are needed to increase vaccine uptake among adolescent and pediatric populations.

Introduction

Severe Acute Respiratory Syndrome (SARS-CoV-2) has caused more than 6.5 million deaths globally [1]. Safe and effective vaccines to prevent SARS-CoV-2 infection and severe outcomes have been approved since late 2020 for adults [2]. With the emergence of viral variants B.1.617.2 (Delta) in May 2021 and B.1.1.529 (Omicron) in late November 2021, decreased vaccine effectiveness against infection was observed [3–6]. Given that the goal of vaccination is to prevent death, severe disease, and overall disease burden, it is important to consider how well vaccines achieve these goals among individuals infected with SARS-CoV-2 [7].

Four recent studies focused on quantifying the real-world effectiveness of SARS-CoV-2 vaccination against hospitalization among adolescent or pediatric populations [8–11]. All the studies used uninfected controls, and resulting vaccine effectiveness estimates reflect the joint risk of SARS-CoV-2 infection and the risk of hospitalization conditional on infection [12]. Olson et al. [8] showed that BNT162b2 was 94% effective against hospitalization among adolescents infected with the Delta variant (B.1.617.2). After the emergence of the Omicron variant (B.1.1.529), two dose vaccine effectiveness against hospitalization was estimated to be 73% among adolescents ages 12 to 17 and 48% among pediatric patients ages 5 to 11 [9]. In a recent Morbidity and Mortality Weekly Report (MMWR), two dose vaccine effectiveness against hospitalization was between 73% and 94% among adolescent and pediatric populations [10]. In a study among hospitalized patients, two dose vaccine effectiveness against SARS-CoV-2 hospitalization was 93% during the Delta-dominant period and 40% during the Omicron-dominant period among adolescents, and 68% during the Omicron-dominant period among pediatric patients [11]. In contrast to the approach used in these studies, we conditioned upon SARS-CoV-2 infection in our analysis to estimate the direct association between vaccination and hospitalization risk among pediatric and adolescent SARS-CoV-2 cases [13].

The Canadian province of Ontario is a large (population 14.6 million) and diverse jurisdiction with high levels of SARS-CoV-2 vaccine coverage [14]. Approximately 87% of Ontario residents ages 12 to 17 and 53% of Ontario residents ages 5 to 11 received at least one SARS-CoV-2 dose as of January 2022 [15]. Most individuals ages 12–17 were eligible to receive the BNT162b2 (Pfizer-BioNTech, Comirnaty) vaccine beginning on May 28, 2021 [16], followed by ages 5 to 11 on November 28, 2021 [17]. Health Canada authorized Moderna Spikevax on August 27, 2021 for ages 12 to 17, but it was not authorized for ages 6 to 11 until after our study period [18]. We calculated one dose and two dose mRNA vaccine effectiveness against hospitalization among adolescent and pediatric SARS-CoV-2 cases to examine if vaccines prevent hospitalization when they fail to prevent SARS-CoV-2 infection.

Methods

Data

Robust public health surveillance systems in Ontario enable individual-level linkage of the SARS-CoV-2 vaccination database and the reportable disease database. Confirmed

SARS-CoV-2 cases were identified in Ontario's Public Health Case and Contact Management Solution (CCM). The CCM includes patient demographics (i.e., sex, age, comorbidities), geographic location (i.e., health region [5 units at the sub-provincial level]), and case characteristics (i.e., test date, symptom onset date, hospital admission and discharge dates) for all laboratory-confirmed SARS-CoV-2 cases in Ontario. SARS-CoV-2 vaccination information was identified from the provincial COVaxON database. COVaxON data includes vaccine administration information (i.e., dose dates, dose locations, dose indication, vaccine product) for Ontario residents with a provincial health insurance number. We linked data from CCM and COVaxON using a unique pseudo identifier present in both datasets. Median household income and percent visible minority at the census subdivision level were from the 2016 Census of Population [19].

Study design

We conducted a retrospective cohort study of individuals aged 4–17 years who had a confirmed SARS-CoV-2 infection in Ontario, Canada. These individuals had a positive reverse transcription real-time polymerase chain reaction (PCR) test between May 28, 2021 and December 4, 2021 (pre-Omicron), or between December 23, 2021 and January 9, 2022 (Omicron). Our study period ended because Ontario restricted publicly funded PCR testing to only select members of the population [20].

Our analyses only included incident SARS-CoV-2 infections (re-infections were excluded). We excluded SARS-CoV-2 cases where individuals had received a vaccine not approved for use in this population in Canada (e.g., Johnson & Johnson/Janssen COVID-19 vaccine, Oxford-AstraZeneca COVID-19 vaccine), or had received three vaccine doses (ages 12–17) or two vaccine doses (ages 4–11). During the study period, adolescents were not eligible for third doses (i.e., booster dose), and the recommended interval between first and second doses for children was eight weeks [21]. We extracted the data on January 19, 2022, but only included test dates up to January 9, 2022, to account for delays between testing, hospitalization, and reporting. SARS-CoV-2 cases aged 4 years were included in the analysis because cases among pediatric patients aged 4 and aged 5 were in the same 2-year age group in these data, and pediatric patients who turned 5 in the 2021 calendar year were eligible for vaccination.

Measures

The outcome was hospitalization due to SARS-CoV-2. Hospitalizations were identified by a reported hospital admission date, or a reported hospitalization or ICU admission due to SARS-CoV-2 in the CCM. The exposure was SARS-CoV-2 vaccination. We considered individuals one-dose vaccinated 14 or more days after the date the first vaccine dose was administered; individuals were considered two-dose vaccinated 14 or more days after the date the second vaccine dose was administered. Individuals infected with SARS-CoV-2 within 13 days of being vaccinated were excluded from our analyses.

Case onset date, age, sex, asthma, immunocompromising condition, and health region were included as confounders [11, 22]. A Directed Acyclic Graph (DAG) outlining the main hypothesized relationships between the variables is presented in **S1 Fig** [23]. Case onset date was the date of symptom onset for symptomatic cases and the specimen collection date for asymptomatic cases. In the CCM, age was reported in 2-year age groups, and asthma and immunocompromising condition were reported by the patient or provider. Health region included northern, eastern, central, or western Ontario, or Toronto [24]. Median household income and percent visible minority in 2016 at the census subdivision level were included as confounders in a sensitivity analysis.

Statistical analysis

Standardized differences (SD) [25, 26] and statistical tests (Fisher's exact tests and unpaired t-tests) were used to compare differences in baseline characteristics between hospitalized and non-hospitalized cases. Standardized mean differences are not impacted by sample size, in contrast to the common statistical tests used in descriptive tables. A standardized difference greater than 0.10 represents a lack of covariate balance [27].

We used multivariable logistic regression to calculate the association between vaccination and hospitalization among adolescent (ages 12–17) and pediatric (ages 4–11) SARS-CoV-2 cases, while adjusting for case onset date, age, sex, asthma, immunocompromising condition, and health region. We examined one-dose and two-dose vaccine effectiveness among the adolescent population prior to the emergence (May 28, 2021 to December 4, 2021) and after the emergence of Omicron (December 23, 2021 to January 9, 2022), and one-dose vaccine effectiveness among the pediatric population after the emergence of Omicron. Ontario residents under age 12 were ineligible for SARS-CoV-2 vaccination during most of the pre-Omicron time period. Dates were chosen to align with the rise in the prevalence of the Omicron variant in Ontario. In the pre-Omicron period, less than 10% of SARS-CoV-2 samples had S-gene target failure and in the latter period, more than 90% of SARS-CoV-2 samples had S-gene target failure [28]. Vaccine effectiveness (VE) was calculated using the formula VE = (1-aOR)*100%.

We performed additional analyses to quantify the impact of unmeasured confounding and to explore the robustness of our results to our assumptions. First, we calculated E-values based on the results of our multivariable logistic regression models [29]. An E-value quantifies how strong an unmeasured confounder would need to be to explain away the association between SARS-CoV-2 vaccination and hospitalization, conditional on other measured covariates [30]. We calculated an E-values using the following formula [29]: E-value = $OR + \sqrt{OR*(1 - OR)}$. Although there is no accepted threshold in the literature for an E-value that demonstrates robustness to unmeasured confounding [31], an E-value is a useful measure to quantify the strength of unmeasured confounding needed to alter our findings [32]. We conducted four model-based sensitivity analyses. First, we allowed for a 14-day delay between case onset date and hospitalization, where SARS-CoV-2 cases with onset dates after January 5, 2022 were excluded. In our main analysis, we allowed for a 10-day delay between case onset date and hospitalization. Second, we repeated our analyses with adjustment for specimen collection date as opposed to case onset date to lessen the impact of differential case onset dates by symptom status. Third, we only included symptomatic individuals as opposed to all SARS-CoV-2 cases. Fourth, we examined the impact of including median household income and percent visible minority at the census subdivision level as confounders in addition to sex, asthma, immunocompromising condition, age, and case onset date in a multivariable logistic regression model. Individual-level measures of socioeconomic status and race were not available in our data and are therefore unmeasured confounders in our study [11]. This study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guideline.

Ethics

We received ethics approval for this study from the Research Ethics Board at the University of Toronto (#00031358). This study includes secondary analysis of public health surveillance data collected under Ontario's Health Protection and Promotion Act. Therefore, the need for informed consent was waived by the ethics committee.

Results

In total, 62 hospitalized SARS-CoV-2 cases and 27,674 non-hospitalized SARS-CoV-2 cases were included (**Fig 1**; **Table 1**). In the full cohort across both age groups and the two time

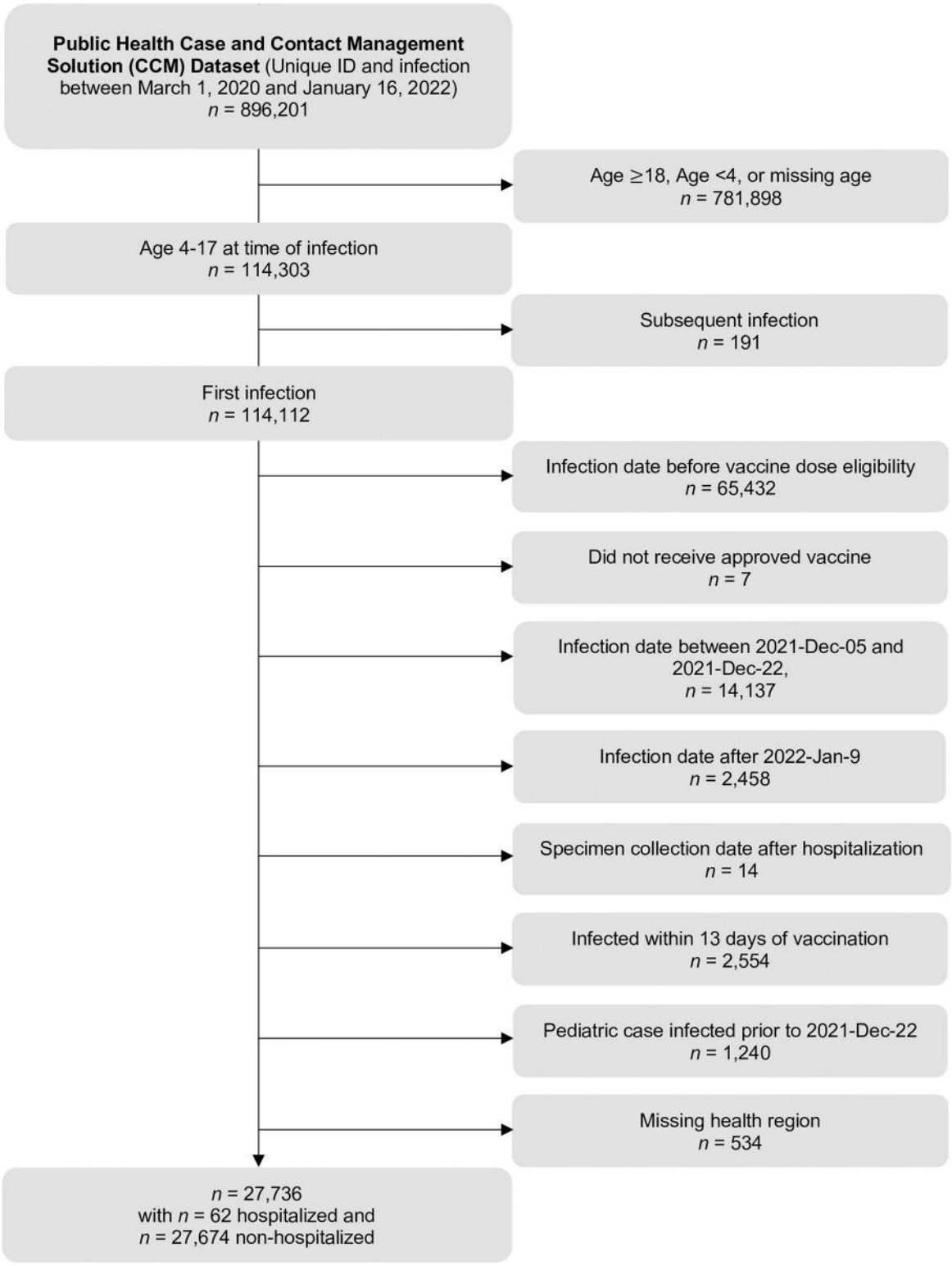

Fig 1. Flow diagram for creation of cohort.

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Table 1. Description of adolescent and pediatric SARS-CoV-2 cases by hospitalization status (n = 27,736).

Characteristic	Full Cohort					
	Hospitalized		Non-hospitalized		SD	p^a
	n = 62		n = 27,674			
	n	(%)	n	(%)		
Vaccination Status[b]					0.73	<0.001
Unvaccinated	47	(75.8)	11,716	(42.3)		
One dose	5	(8.1)	4,749	(17.2)		
Two doses	10	(16.1)	11,209	(40.5)		
Male					0.03	0.84
Yes	30	(48.4)	13,756	(49.7)		
No	32	(51.6)	13,918	(50.3)		
Age					0.30	0.51
4–5 years	5	(8.1)	2,100	(7.6)		
6–7 years	6	(9.7)	2,613	(9.4)		
8–9 years	5	(8.1)	3,006	(10.9)		
10–11 years	4	(6.5)	3,334	(12.0)		
12–13 years	10	(16.1)	4,715	(17.0)		
14–15 years	10	(16.1)	5,102	(18.4)		
16–17 years	22	(35.5)	6,804	(24.6)		
Immunocompromised					0.50	<0.001
Yes	7	(11.3)	40	(0.01)		
No	55	(88.7)	27,634	(99.9)		
Asthma					0.33	<0.001
Yes	4	(6.5)	143	(5.0)		
No	58	(93.5)	27,491	(99.5)		
Region					0.45	<0.01
Central	16	(25.8)	10,378	(37.5)		
East	16	(25.8)	6,134	(22.2)		
North	8	(12.9)	1,208	(4.4)		
Toronto	2	(3.2)	2,414	(8.7)		
West	20	(32.3)	7,540	(27.2)		
	Mean	**(Std)**	**Mean**	**(Std)**		
Case Onset Date[c]	2021-10-26	(82 days)	2021-12-10	(48 days)	0.66	<0.001

Notes: SD = standardized difference; Std = standard deviation

[a] Fischer's exact test for categorical variables, and unpaired t-tests for continuous variables

[b] Vaccination status on case onset date

[c] Date of symptom onset for symptomatic cases and the specimen collection date for asymptomatic cases

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periods, individuals hospitalized with SARS-CoV-2 were more likely to be unvaccinated, immunocompromised, have an asthma diagnosis, live in the West or North health regions, and have an earlier case onset date (Table 1). We were unable to present frequencies, percentages, means, and standard deviations for the cohort stratified by age group and time period due to small cell sizes, however the relationships were quantified using standardized differences and statistical tests (S1 Table).

In the pre-Omicron period after adjustment for sex, asthma, immunocompromising condition, age, health region, and case onset date, we observed that one and two SARS-CoV-2 vaccine doses were not significantly associated (adjusted OR (aOR) one dose = 3.09 [95% CI: 0.31,

Table 2. Adjusted odds ratios of the association between vaccination status and hospitalization among adolescent and pediatric SARS-CoV-2 cases (n = 27,736).

Characteristic	Adolescent						Pediatric		
	Pre-Omicron			Omicron			Omicron		
	2021-May-28 to 2021-Dec-05 n = 4,999 (30 hospitalized)			2021-Dec-23 to 2022-Jan-09 n = 11,664 (12 hospitalized)			2021-Dec-23 to 2022-Jan-09 n = 11,073 (20 hospitalized)		
	aOR	(95% CI)	p	aOR	(95% CI)	p	aOR	(95% CI)	p
Vaccination Status[b]									
Unvaccinated	1.00	(ref)		1.00	(ref)		1.00	(ref)	
One dose	3.09	(0.31, 16.84)	0.26	0.90	(0.05, 5.83)	0.93	0.21	(0.03, 0.77)	<0.05
Two doses	0.90	(0.22, 2.86)	0.87	0.15	(0.04, 0.53)	<0.01	--	--	
Male									
Yes	0.81	(0.37, 1.71)	0.58	1.81	(0.57, 6.33)	0.32	0.71	(0.28, 1.75)	0.45
No	1.00	(ref)		1.00	(ref)		1.00	(ref)	
Age[c]	1.36	(1.07, 1.78)	<0.05	0.94	(0.66, 1.35)	0.74	0.92	(0.75, 1.13)	0.43
Immunocompromised									
Yes	126.16	(32.05, 462.37)	<0.001	--[a]	--		107.91	(14.52, 528.88)	<0.001
No	1.00	(ref)		1.00	(ref)		1.00	(ref)	
Asthma									
Yes	4.24	(0.67, 15.03)	0.06	40.22	(1.90, 295.74)	<0.01	28.65	(1.44, 177.65)	<0.01
No	1.00	(ref)		1.00	(ref)		1.00	(ref)	
Region									
Central	1.00	(ref)		1.00	(ref)		1.00	(ref)	
East	1.05	(0.30, 3.30)	0.93	4.64	(1.03, 32.10)	0.06	1.54	(0.44, 5.21)	0.48
North	2.74	(0.71, 9.12)	0.11	7.56	(0.89, 64.23)	<0.05 [d]	3.32	(0.46, 15.40)	0.16
Toronto	0.25	(0.01, 1.76)	0.24	--[a]	--		0.75	(0.04, 4.54)	0.79
West	1.11	(0.44, 2.98)	0.82	1.36	(0.16, 11.49)	0.76	1.75	(0.53, 5.76)	0.34
Case Onset Date[d]	0.99	(0.98, 1.00)	<0.01	1.14	(1.00, 1.29)	<0.05	1.22	(1.11, 1.36)	<0.001

[a] No individuals who were immunocompromised or in the Toronto health region were included in this analysis due to too few hospitalized cases with these characteristics

[b] Vaccination status on case onset date

[c] Age as continuous

[d] Date of symptom onset for symptomatic cases and the specimen collection date for asymptomatic cases; [d] Discrepancy in significance is due to comparing a likelihood-based 95% CI and a Wald p-value

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16.84], p = 0.26; aOR two doses = 0.90 [95% CI: 0.22, 2.86), p = 0.15) with additional protection against hospitalization among adolescent SARS-CoV-2 cases (Table 2). In the Omicron period after adjustment for sex, asthma, immunocompromising condition, age, health region, and case onset date, one SARS-CoV-2 dose was not significantly associated (aOR = 0.90 [95% CI: 0.05, 5.83], p = 0.93) with additional protection against hospitalization, but two SARS-CoV-2 doses were associated with an 85% (aOR = 0.15, [95% CI: 0.04, 0.53], p<0.01) lower likelihood of hospitalization among adolescent SARS-CoV-2 cases. After adjustment for sex, asthma, immunocompromising condition, age, health region, and case onset date, one SARS-CoV-2 vaccine dose was associated with a 79% (aOR = 0.21, [95% CI: 0.03, 0.77], p<0.05) lower likelihood of hospitalization among pediatric SARS-CoV-2 cases (ages 4–11) in the Omicron period (Table 2).

The E-value for the adjusted association between vaccination with two doses and the risk of hospitalization in our primary analysis among the adolescent population in the Omicron period (i.e., aOR = 0.15) was 12.8 [29]. An unmeasured confounder would need to be independently associated with vaccination and hospitalization by a 12.8-fold risk ratio to result in a

null association, after adjusting for sex, asthma, immunocompromising condition, age, health region, and case onset date. Similarly, the E-value for the upper 95% CI bound (i.e., 95% CI upper bound = 0.53) is 3.2. A set of unmeasured confounders would need to be independently associated with vaccination and hospitalization by a 3.2-fold risk ratio for the 95% CI to encompass the null value after controlling sex, asthma, immunocompromising condition, age, health region, and case onset date. The E-value for the adjusted association between vaccination with one dose and the risk of hospitalization among the pediatric population (i.e., aOR = 0.21) was 9.0 and the E-value for the upper 95% CI bound (i.e., 95% CI upper bound = 0.77) was 1.9.

Our vaccine effectiveness estimates did not substantially change when we matched on specimen collection date, when we only included symptomatic cases, and when we included income and visible minority status at the census subdivision level (**S2 Table**). In our sensitivity analysis where only cases with onset dates prior to January 5, 2022 were included, the association between one vaccine dose and hospitalization among pediatric Omicron SARS-CoV-2 cases was invalid (due to too few hospitalizations in the time period), but the association among adolescent Omicron SARS-CoV-2 cases remained unchanged compared to our main analysis (**S2 Table**).

Discussion

In this population-based analysis from the Canadian province of Ontario, we find evidence that mRNA vaccines against SARS-CoV-2 prevent hospitalization when adolescents receive a full two dose series and children received one dose in the context of an epidemic dominated by the immune-evasive Omicron variants. Among adolescent SARS-CoV-2 cases one vaccine dose was not significantly associated with a lower likelihood of protection against hospitalization in the pre-Omicron and Omicron periods, and two doses were not significantly protective in the pre-Omicron period. Our vaccine effectiveness estimates demonstrate robustness to unmeasured confounding, as evidenced by the high E-values for the estimates. As all subjects in this study had SARS-CoV-2 infection, the protection afforded by vaccination against hospitalization relates to attenuation of disease and is independent of protection provided against infection. This may in part explain our finding that vaccination was ineffective at preventing hospitalization in adolescents conditional upon infection with the Delta variant. SARS-CoV-2 infection with the Delta variant was less common among vaccinated individuals, and our study cohort during the Delta time period likely had a higher proportion of high risk population members compared to during the Omicron period [33].

The studies that have focused on SARS-CoV-2 vaccine effectiveness against hospitalization in adolescent and pediatric populations are not directly comparable to ours due to differences in control selection. Our estimated vaccine effectiveness estimates against hospitalization are lower than most estimates from studies using negative controls in the United States [8–10], likely because we isolated the impact of vaccination of hospitalization risk, independent of susceptibility to SARS-CoV-2 infection. In a case-control study conducted between May and October 2021 (i.e., prior to widespread infection with Omicron) with test-negative and syndrome-negative controls, vaccine effectiveness against hospitalization was 94% among those with two doses and 97% among those with one dose [8]. A study with 164 hospitals in the United States from April 2021 to January 2022 found that two dose vaccine effectiveness against hospitalization was 74% among children ages 5–11 years and between 73% and 94% among adolescents [10].

In contrast, two dose vaccine effectiveness was calculated to be 40% against hospitalization among adolescents during the circulation of Omicron in a study including 31 hospitals across

the United States [11]. This lower estimate but may be due to earlier adolescent vaccination dates in the United States compared to in Ontario, as well as the impact of vaccine waning [11]. Unlike prior studies, our control group consisted of non-hospitalized SARS-CoV-2 cases, not uninfected individuals. This allowed for the isolation of the effectiveness of vaccination against hospitalization independent of infection risk, which explains our lower vaccine effectiveness estimates against hospitalization compared to the majority of prior studies [12].

Our study has several notable strengths. Due to Ontario's high quality public health surveillance data, we isolated the impact of vaccination on hospitalization risk with control for individual level demographic and health related factors. Our study includes the population of a diverse region with publicly funded healthcare [34, 35]. We used a quantitative bias analysis to quantify the susceptibility of our results to unmeasured confounding [29]. Our study also had a few limitations. First, we did not consider time since vaccination in our analysis. In a recent study in the United States, time since vaccination was not shown to significantly impact SARS-CoV-2 vaccine effectiveness estimates against hospitalization in younger individuals [10]. We were unable to assess two dose effectiveness against hospitalization specifically among cases ages 4–11 years due to few hospitalizations among two-dose vaccinated individuals. Additionally, with only 62 total hospitalizations included in our study, we were likely underpowered to detect all true vaccine effects. Vaccination may be protective where our study showed a lack of statistical significance (i.e., Type II error), and a larger study over a longer period is needed. Some individuals aged four in our study were ineligible for vaccination. If they had a higher incidence of hospitalization, our vaccine effectiveness estimates would be biased away from the null. The availability of testing, and the propensity to get tested may have differed between vaccinated and unvaccinated individuals, and the comorbidities may have been more completely ascertained among hospitalized SARS-CoV-2 cases. Finally, comorbidities (i.e., asthma and immunocompromising conditions) in the CCM did not have a standardized definition which leads to residual confounding.

With the continued emergence of variants that may further decrease SARS-CoV-2 vaccine effectiveness against infection, it is vital to consider how effective these vaccines are at preventing severe outcomes [36]. Future studies should examine the impact of subsequent vaccine doses in adolescent and pediatric populations on SARS-CoV-2 hospitalization risk with emerging variants.

Conclusions

In this evaluation of the effectiveness of SARS-CoV-2 vaccination in adolescent and pediatric Omicron cases ages 4 to 17 in Ontario, Canada, we found that vaccination with two doses among adolescents and one dose among children is associated with a decreased likelihood of hospitalization, even when the vaccines do not prevent infection. SARS-CoV-2 vaccines remain an effective intervention to prevent severe outcomes in adolescent and pediatric populations, and continued efforts are needed to increase vaccine uptake in these populations.

Supporting information

S1 Fig. Directed Acyclic Graph (DAG). DAG of the relationship between vaccination status and hospitalization among adolescent and pediatric SARS-CoV-2 cases.
(TIF)

S1 Table. Description of adolescent and pediatric SARS-CoV-2 cases by hospitalization status, stratified by age and time period (*n* = 27,736).
(DOCX)

S2 Table. Adjusted odds ratios of the association between vaccination status and hospitalization among adolescent and pediatric SARS-CoV-2 cases with varying assumptions.
(DOCX)

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This is Exhibit I referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Original research

The role of COVID-19 vaccines in preventing post-COVID-19 thromboembolic and cardiovascular complications

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ABSTRACT

Objective To study the association between COVID-19 vaccination and the risk of post-COVID-19 cardiac and thromboembolic complications.

Methods We conducted a staggered cohort study based on national vaccination campaigns using electronic health records from the UK, Spain and Estonia. Vaccine rollout was grouped into four stages with predefined enrolment periods. Each stage included all individuals eligible for vaccination, with no previous SARS-CoV-2 infection or COVID-19 vaccine at the start date. Vaccination status was used as a time-varying exposure. Outcomes included heart failure (HF), venous thromboembolism (VTE) and arterial thrombosis/thromboembolism (ATE) recorded in four time windows after SARS-CoV-2 infection: 0–30, 31–90, 91–180 and 181–365 days. Propensity score overlap weighting and empirical calibration were used to minimise observed and unobserved confounding, respectively. Fine-Gray models estimated subdistribution hazard ratios (sHR). Random effect meta-analyses were conducted across staggered cohorts and databases.

Results The study included 10.17 million vaccinated and 10.39 million unvaccinated people. Vaccination was associated with reduced risks of acute (30-day) and post-acute COVID-19 VTE, ATE and HF: for example, meta-analytic sHR of 0.22 (95% CI 0.17 to 0.29), 0.53 (0.44 to 0.63) and 0.45 (0.38 to 0.53), respectively, for 0–30 days after SARS-CoV-2 infection, while in the 91–180 days sHR were 0.53 (0.40 to 0.70), 0.72 (0.58 to 0.88) and 0.61 (0.51 to 0.73), respectively.

Conclusions COVID-19 vaccination reduced the risk of post-COVID-19 cardiac and thromboembolic outcomes. These effects were more pronounced for acute COVID-19 outcomes, consistent with known reductions in disease severity following breakthrough versus unvaccinated SARS-CoV-2 infection.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ COVID-19 vaccines proved to be highly effective in reducing the severity of acute SARS-CoV-2 infection.
⇒ While COVID-19 vaccines were associated with increased risk for cardiac and thromboembolic events, such as myocarditis and thrombosis, the risk of complications was substantially higher due to SARS-CoV-2 infection.

WHAT THIS STUDY ADDS

⇒ COVID-19 vaccination reduced the risk of heart failure, venous thromboembolism and arterial thrombosis/thromboembolism in the acute (30 days) and post-acute (31 to 365 days) phase following SARS-CoV-2 infection. This effect was stronger in the acute phase.
⇒ The overall additive effect of vaccination on the risk of post-vaccine and/or post-COVID thromboembolic and cardiac events needs further research.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ COVID-19 vaccines proved to be highly effective in reducing the risk of post-COVID cardiovascular and thromboembolic complications.

INTRODUCTION

COVID-19 vaccines were approved under emergency authorisation in December 2020 and showed high effectiveness against SARS-CoV-2 infection, COVID-19-related hospitalisation and death.[1 2] However, concerns were raised after spontaneous reports of unusual thromboembolic events following adenovirus-based COVID-19 vaccines, an association that was further assessed in observational studies.[3 4] More recently, mRNA-based vaccines were found to be associated with a risk of rare myocarditis events.[5 6]

On the other hand, SARS-CoV-2 infection can trigger cardiac and thromboembolic complications.[7 8] Previous studies showed that, while slowly decreasing over time, the risk for serious complications remain high for up to a year after infection.[9 10] Although acute and post-acute cardiac and thromboembolic complications following COVID-19 are rare, they present a substantial burden to the

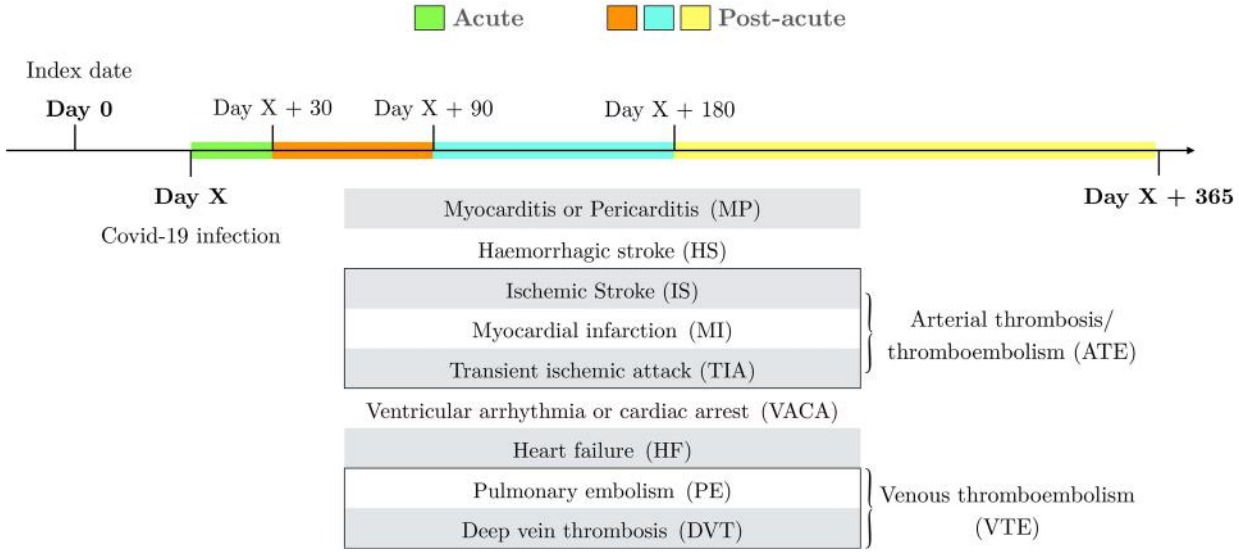

Figure 1 Study outcome design. Study outcomes of interest are defined as a COVID-19 infection followed by one of the complications in the figure, within a year after infection. Outcomes were ascertained in four different time windows after SARS-CoV-2 infection: 0–30 days (namely the acute phase), 31–90 days, 91–180 days and 181–365 days (these last three comprise the post-acute phase).

affected patients, and the absolute number of cases globally could become substantial.

Recent studies suggest that COVID-19 vaccination could protect against cardiac and thromboembolic complications attributable to COVID-19.[11 12] However, most studies did not include long-term complications and were conducted among specific populations.

Evidence is still scarce as to whether the combined effects of COVID-19 vaccines protecting against SARS-CoV-2 infection and reducing post-COVID-19 cardiac and thromboembolic outcomes, outweigh any risks of these complications potentially associated with vaccination.

We therefore used large, representative data sources from three European countries to assess the overall effect of COVID-19 vaccines on the risk of acute and post-acute COVID-19 complications including venous thromboembolism (VTE), arterial thrombosis/thromboembolism (ATE) and other cardiac events. Additionally, we studied the comparative effects of ChAdOx1 versus BNT162b2 on the risk of these same outcomes.

METHODS

Data sources

We used four routinely collected population-based healthcare datasets from three European countries: the UK, Spain and Estonia.

For the UK, we used data from two primary care databases—namely, Clinical Practice Research Datalink, CPRD Aurum[13] and CPRD Gold.[14] CPRD Aurum currently covers 13 million people from predominantly English practices, while CPRD Gold comprises 3.1 million active participants mostly from GP practices in Wales and Scotland. Spanish data were provided by the Information System for the Development of Research in Primary Care (SIDIAP),[15] which encompasses primary care records from 6 million active patients (around 75% of the population in the region of Catalonia) linked to hospital admissions data (Conjunt Mínim Bàsic de Dades d'Alta Hospitalària). Finally, the CORIVA dataset based on national health claims data from Estonia was used. It contains all COVID-19 cases from the first year of the pandemic and ~440 000 randomly selected controls. CORIVA was linked to the death registry and all COVID-19 testing from the national health information system.

Databases included sociodemographic information, diagnoses, measurements, prescriptions and secondary care referrals and were linked to vaccine registries, including records of all administered vaccines from all healthcare settings. Data availability for CPRD Gold ended in December 2021, CPRD Aurum in January 2022, SIDIAP in June 2022 and CORIVA in December 2022.

All databases were mapped to the Observational Medical Outcomes Partnership Common Data Model (OMOP CDM)[16] to facilitate federated analytics.

Multinational network staggered cohort study: study design and participants

The study design has been published in detail elsewhere.[17] Briefly, we used a staggered cohort design considering vaccination as a time-varying exposure. Four staggered cohorts were designed with each cohort representing a country-specific vaccination rollout phase (eg, dates when people became eligible for vaccination, and eligibility criteria).

The source population comprised all adults registered in the respective database for at least 180 days at the start of the study (4 January 2021 for CPRD Gold and Aurum, 20 February 2021 for SIDIAP and 28 January 2021 for CORIVA). Subsequently, each staggered cohort corresponded to an enrolment period: all people eligible for vaccination during this time were included in the cohort and people with a history of SARS-CoV-2 infection or COVID-19 vaccination before the start of the enrolment period were excluded. Across countries, cohort 1 comprised older age groups, whereas cohort 2 comprised individuals at risk for severe COVID-19. Cohort 3 included people aged ≥40 and cohort 4 enrolled people aged ≥18.

In each cohort, people receiving a first vaccine dose during the enrolment period were allocated to the vaccinated group, with their index date being the date of vaccination. Individuals who did not receive a vaccine dose comprised the unvaccinated group and their index date was assigned within the enrolment period, based on the distribution of index dates in the vaccinated group. People with COVID-19 before the index date were excluded.

Table 1 Characteristics of weighted populations in CPRD Aurum database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine

Characteristics	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	Vaccinated	ASMD	Unvaccinated	Vaccinated	ASMD	Unvaccinated	Vaccinated	ASMD	Unvaccinated	Vaccinated	ASMD
No (individuals)	154 864	154 245		420 707	420 931		463 495	462 463		818 917	827 124	
Age, median (Q25–Q75)	80 (76–84)	80 (76–84)	0.000	58 (44–67)	58 (44–67)	0.005	50 (41–58)	52 (40–58)	0.003	34 (26–42)	34 (26–42)	0.004
Sex: female, N (%)	88 349 (57%)	87 639 (57%)	0.005	248 156 (59%)	249 561 (59%)	0.006	245 248 (53%)	245 600 (53%)	0.004	351 435 (43%)	358 688 (43%)	0.009
Years of prior history*, median (Q25–Q75)	24 (10–35)	24 (10–36)	0.006	18 (8–29)	18 (8–29)	0.003	14 (6–24)	14 (7–24)	0.008	8 (4–17)	7 (3–18)	0.001
Number of GP visits, median (Q25–Q75)	10 (5–18)	10 (6–17)		8 (3–15)	8 (5–14)		4 (1–11)	6(3-11)		2 (0–6)	2 (0–6)	
Number of PCR tests, median (Q25–Q75)	0 (0–0)	0 (0–0)		0 (0–0)	0 (0–0)		0 (0–0)	0 (0–0)		0 (0–0)	0 (0–0)	
Comorbiditiest, N (%)												
Anxiety	23 200 (15%)	22 789 (15%)	0.006	94 390 (22%)	91 644 (22%)	0.016	92 820 (20%)	90 807 (20%)	0.010	123 055 (15%)	125 202 (15%)	0.003
Asthma	16 978 (11%)	16 663 (11%)	0.005	95 770 (23%)	94 550 (22%)	0.007	79 642 (17%)	78 266 (17%)	0.007	63 687 (8%)	61 472 (7%)	0.013
Chronic kidney disease	36 149 (23%)	36 046 (23%)	0.001	28 181 (7%)	29 756 (7%)	0.015	10 283 (2%)	10 577 (2%)	0.005	3840 (0%)	3572 (0%)	0.006
COPD	13 385 (9%)	13 181 (9%)	0.003	17 447 (4%)	17 999 (4%)	0.006	6062 (1%)	5754 (1%)	0.006	1901 (0%)	1918 (0%)	0.000
Dementia	9483 (6%)	8517 (6%)	0.026	4182 (1%)	3879 (1%)	0.007	1361 (0%)	1392 (0%)	0.001	276 (0%)	495 (0%)	0.012
Depressive disorder	18 632 (12%)	18 547 (12%)	0.000	85 280 (20%)	81 945 (19%)	0.020	81 891 (18%)	79 804 (17%)	0.011	94 373 (12%)	97 053 (12%)	0.007
Diabetes	29 365 (19%)	28 831 (19%)	0.007	49 408 (12%)	48 562 (12%)	0.006	26 616 (6%)	28 628 (6%)	0.019	12 787 (2%)	12 539 (2%)	0.004
GORD	8718 (6%)	8515 (6%)	0.005	19 907 (5%)	18 924 (4%)	0.011	15 646 (3%)	14 982 (3%)	0.008	13 882 (2%)	13 893 (2%)	0.001
Heart failure	9349 (6%)	8851 (6%)	0.013	7284 (2%)	6502 (2%)	0.015	2660 (1%)	2470 (1%)	0.005	930 (0%)	816 (0%)	0.005
Hypertension	81 563 (53%)	80 806 (52%)	0.006	97 707 (23%)	98 193 (23%)	0.002	54 649 (12%)	55 798 (12%)	0.008	22 925 (3%)	24 450 (3%)	0.009
Hypothyroidism	15 125 (10%)	15 098 (10%)	0.001	25 579 (6%)	25 962 (6%)	0.004	17 162 (4%)	17 580 (4%)	0.005	12 427 (2%)	12 641 (2%)	0.001
Malignant neoplastic disease	33 467 (22%)	33 024 (21%)	0.005	30 194 (7%)	35 085 (8%)	0.043	14 815 (3%)	14 140 (3%)	0.008	6447 (1%)	5766 (1%)	0.011
Myocardial infarction	7824 (5%)	7731 (5%)	0.002	9964 (2%)	11 319 (3%)	0.020	3787 (1%)	3664 (1%)	0.003	1315 (0%)	1069 (0%)	0.008
Osteoporosis	15 275 (10%)	15 373 (10%)	0.003	10 626 (3%)	10 718 (3%)	0.001	4113 (1%)	4131 (1%)	0.001	1376 (0%)	1472 (0%)	0.002
Pneumonia	8573 (6%)	7621 (5%)	0.027	11 355 (3%)	10 691 (3%)	0.010	6651 (1%)	6545 (1%)	0.002	5144 (1%)	5151 (1%)	0.001
Rheumatoid arthritis	3066 (2%)	3092 (2%)	0.002	6198 (1%)	6570 (2%)	0.007	2355 (1%)	3111 (1%)	0.021	1201 (0%)	859 (0%)	0.012
Stroke	7667 (5%)	7047 (5%)	0.018	8041 (2%)	8794 (2%)	0.013	3518 (1%)	3293 (1%)	0.006	1496 (0%)	1305 (0%)	0.006
Venous thromboembolism	9589 (6%)	9241 (6%)	0.008	11 836 (3%)	12 475 (3%)	0.009	6503 (1%)	8075 (2%)	0.028	4661 (1%)	2441 (0%)	0.042

The four cohorts represent vaccine rollout periods.
*Calculated as the days of previous observation in the database before index date.
†Assessed any time before index date.
ASMD, absolute standardised mean difference; COPD, chronic obstructive pulmonary disease; GORD, gastro-oesophageal reflux disease; GP, general practitioner; PCR, polymerase chain reaction.

Table 2 Number of records (and risk per 10 000 individuals) for acute and post-acute COVID-19 cardiac and thromboembolic complications, across cohorts and databases for any COVID-19 vaccination

			CPRD Aurum		CORIVA		CPRD Gold		SIDIAP	
Cohort	Time window	Outcome	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			n=346 674	n=552 602	n=23 982	n=26 736	n=169 100	n=118 507	n=223 962	n=89 941
	0 to 30 days	**VTE**	93 (2.68)	117 (2.12)	77 (32.11)	45 (16.83)	8 (0.47)	8 (0.68)	74 (3.30)	96 (10.67)
		ATE	22 (0.63)	70 (1.27)	110 (45.87)	81 (30.30)	6 (0.35)	7 (0.59)	77 (3.44)	208 (23.13)
		HF	59 (1.70)	198 (3.58)	395 (164.71)	299 (111.83)	10 (0.59)	9 (0.76)	302 (13.48)	640 (71.16)
	31 to 90 days	**VTE**	19 (0.55)	40 (0.72)	37 (15.43)	30 (11.22)	< 5	< 5	16 (0.71)	46 (5.11)
		ATE	5 (0.14)	43 (0.78)	33 (13.76)	47 (17.58)	< 5	< 5	41 (1.83)	130 (14.45)
		HF	30 (0.87)	113 (2.04)	151 (62.96)	170 (63.58)	< 5	8 (0.68)	89 (3.97)	298 (33.13)
	91 to 180 days	**VTE**	10 (0.29)	21 (0.38)	21 (8.76)	35 (13.09)	< 5	< 5	20 (0.89)	40 (4.45)
		ATE	11 (0.32)	28 (0.51)	31 (12.93)	52 (19.45)	< 5	6 (0.51)	30 (1.34)	112 (12.45)
		HF	37 (1.07)	95 (1.72)	162 (67.55)	220 (82.29)	< 5	5 (0.42)	87 (3.88)	252 (28.02)
	181 to 365 days	**VTE**	10 (0.29)	11 (0.20)	45 (18.76)	35 (13.09)	< 5	< 5	10 (0.45)	13 (1.45)
		ATE	10 (0.29)	23 (0.42)	55 (22.93)	82 (30.67)	< 5	< 5	42 (1.88)	53 (5.89)
		HF	40 (1.15)	58 (1.05)	268 (111.75)	321 (120.06)	< 5	6 (0.51)	86 (3.84)	149 (16.57)
Cohort 2			n=1 975 726	n=1 563 569	n=34 317	n=4572	n=583 399	n=486 619	n=433 151	n=819 590
	0 to 30 days	**VTE**	241 (1.22)	220 (1.41)	79 (23.02)	7 (15.31)	31 (0.53)	24 (0.49)	258 (5.96)	400 (4.88)
		ATE	41 (0.21)	104 (0.67)	110 (32.05)	5 (10.94)	< 5	6 (0.12)	173 (3.99)	669 (8.16)
		HF	45 (0.23)	146 (0.93)	364 (106.07)	23 (50.31)	5 (0.09)	13 (0.27)	378 (8.73)	1331 (16.24)
	31 to 90 days	**VTE**	43 (0.22)	76 (0.49)	31 (9.03)	5 (10.94)	< 5	9 (0.18)	59 (1.36)	195 (2.38)
		ATE	18 (0.09)	93 (0.59)	32 (9.32)	< 5	< 5	9 (0.18)	85 (1.96)	444 (5.42)
		HF	27 (0.14)	103 (0.66)	149 (43.42)	19 (41.56)	< 5	7 (0.14)	138 (3.19)	643 (7.85)
	91 to 180 days	**VTE**	28 (0.14)	40 (0.26)	26 (7.58)	6 (13.12)	6 (0.10)	< 5	58 (1.34)	125 (1.53)
		ATE	17 (0.09)	43 (0.28)	32 (9.32)	< 5	< 5	< 5	91 (2.10)	417 (5.09)
		HF	22 (0.11)	69 (0.44)	166 (48.37)	21 (45.93)	< 5	< 5	110 (2.54)	579 (7.06)
	181 to 365 days	**VTE**	9 (0.05)	13 (0.08)	44 (12.82)	8 (17.50)	< 5	< 5	16 (0.37)	64 (0.78)
		ATE	12 (0.06)	18 (0.12)	53 (15.44)	< 5	< 5	< 5	63 (1.45)	178 (2.17)
		HF	20 (0.10)	35 (0.22)	259 (75.47)	33 (72.18)	< 5	< 5	81 (1.87)	246 (3.00)
Cohort 3			n=1 510 401	n=1 528 031	n=96 423	n=24 050	n=417 996	n=462 832	n=869 497	n=954 232
	0 to 30 days	**VTE**	245 (1.62)	142 (0.93)	115 (11.93)	9 (3.74)	27 (0.65)	17 (0.37)	325 (3.74)	180 (1.89)
		ATE	29 (0.19)	49 (0.32)	119 (12.34)	12 (4.99)	< 5	12 (0.26)	213 (2.45)	275 (2.88)
		HF	31 (0.21)	38 (0.25)	380 (39.41)	23 (9.56)	< 5	< 5	364 (4.19)	256 (2.68)
	31 to 90 days	**VTE**	44 (0.29)	46 (0.30)	50 (5.19)	10 (4.16)	< 5	7 (0.15)	85 (0.98)	92 (0.96)
		ATE	11 (0.07)	33 (0.22)	48 (4.98)	9 (3.74)	< 5	8 (0.17)	109 (1.25)	210 (2.20)
		HF	15 (0.10)	26 (0.17)	180 (18.67)	25 (10.40)	< 5	< 5	137 (1.58)	157 (1.65)
	91 to 180 days	**VTE**	24 (0.16)	26 (0.17)	43 (4.46)	11 (4.57)	< 5	< 5	64 (0.74)	101 (1.06)
		ATE	< 5	28 (0.18)	44 (4.56)	10 (4.16)	< 5	< 5	113 (1.30)	206 (2.16)
		HF	11 (0.07)	14 (0.09)	216 (22.40)	30 (12.47)	< 5	< 5	120 (1.38)	138 (1.45)
	181 to 365 days	**VTE**	< 5	11 (0.07)	72 (7.47)	17 (7.07)	< 5	< 5	34 (0.39)	26 (0.27)
		ATE	< 5	< 5	80 (8.30)	8 (3.33)	< 5	< 5	51 (0.59)	67 (0.70)
		HF	5 (0.03)	< 5	324 (33.60)	37 (15.38)	< 5	< 5	62 (0.71)	44 (0.46)
Cohort 4			n=2 027 763	n=2 085 598	n=147 545	n=22 245	n=469 876	n=550 437	n=1 061 634	n=880 950
	0 to 30 days	**VTE**	334 (1.65)	50 (0.24)	116 (7.86)	< 5	36 (0.77)	11 (0.20)	350 (3.30)	98 (1.11)
		ATE	26 (0.13)	8 (0.04)	116 (7.86)	10 (4.50)	< 5	< 5	231 (2.18)	95 (1.08)
		HF	28 (0.14)	< 5	364 (24.67)	17 (7.64)	< 5	< 5	362 (3.41)	75 (0.85)
	31 to 90 days	**VTE**	58 (0.29)	22 (0.11)	54 (3.66)	< 5	5 (0.11)	< 5	91 (0.86)	49 (0.56)
		ATE	12 (0.06)	9 (0.04)	46 (3.12)	5 (2.25)	< 5	< 5	118 (1.11)	76 (0.86)
		HF	14 (0.07)	9 (0.04)	176 (11.93)	13 (5.84)	< 5	< 5	142 (1.34)	47 (0.53)
	91 to 180 days	**VTE**	26 (0.13)	10 (0.05)	49 (3.32)	5 (2.25)	< 5	< 5	71 (0.67)	60 (0.68)
		ATE	< 5	6 (0.03)	41 (2.78)	7 (3.15)	< 5	< 5	128 (1.21)	90 (1.02)
		HF	10 (0.05)	< 5	208 (14.10)	18 (8.09)	< 5	< 5	139 (1.31)	55 (0.62)
	181 to 365 days	**VTE**	< 5	< 5	77 (5.22)	< 5	< 5	< 5	46 (0.43)	12 (0.14)
		ATE	< 5	< 5	73 (4.95)	9 (4.05)	< 5	< 5	54 (0.51)	28 (0.32)
		HF	< 5	< 5	301 (20.40)	16 (7.19)	< 5	< 5	57 (0.54)	15 (0.17)

The four cohorts represent vaccine rollout periods.
ATE, arterial thrombosis/thromboembolism (Ischaemic stroke+transient ischaemic attack+myocardial infarction); HF, heart failure; VTE, venous thromboembolism (deep vein thrombosis+pulmonary embolism).

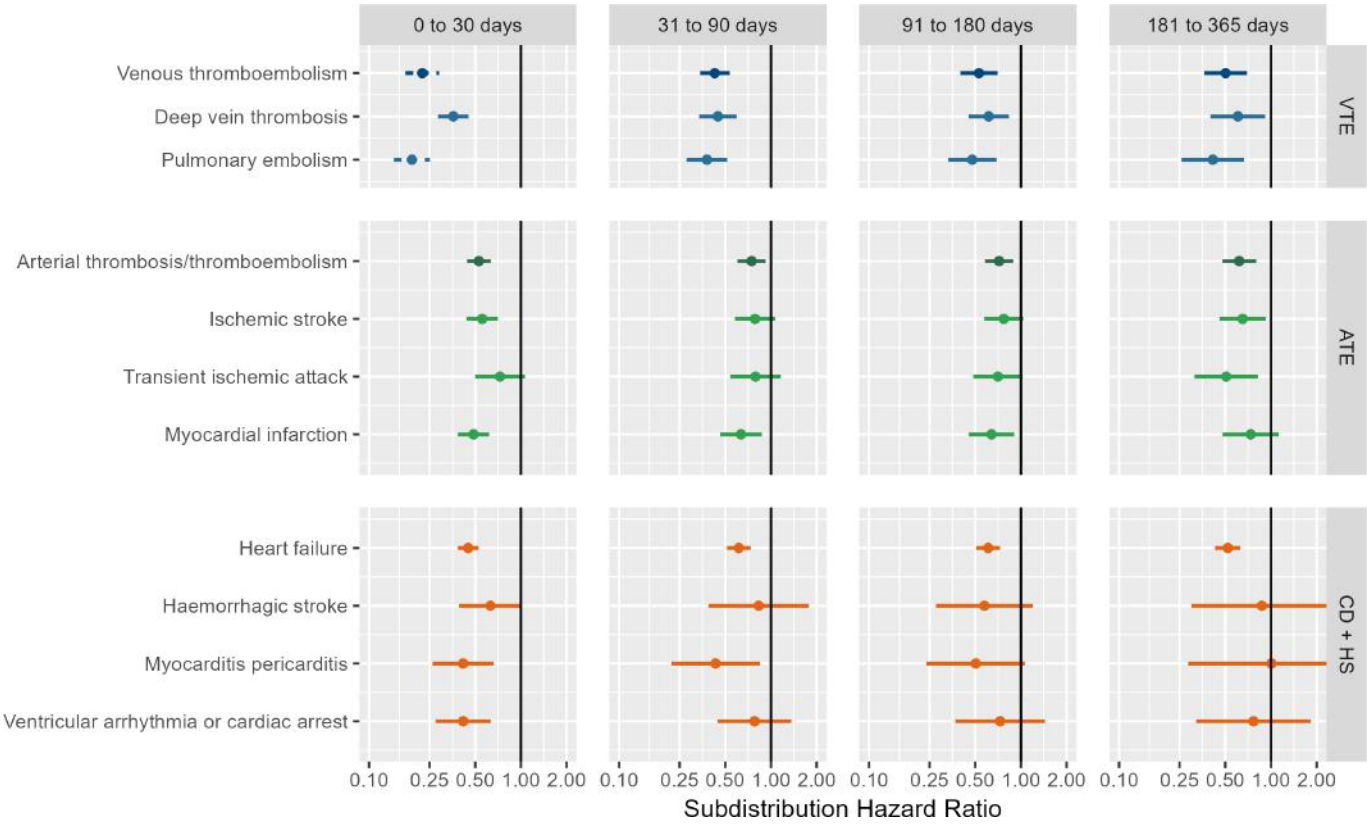

Figure 2 Forest plots for the effect of COVID-19 vaccines on post-COVID-19 cardiac and thromboembolic complications; meta-analysis across cohorts and databases. Dashed line represents a level of heterogeneity I^2>0.4. ATE, arterial thrombosis/thromboembolism; CD+HS, cardiac diseases and haemorrhagic stroke; VTE, venous thromboembolism.

Follow-up started from the index date until the earliest of end of available data, death, change in exposure status (first vaccine dose for those unvaccinated) or outcome of interest.

COVID-19 vaccination

All vaccines approved within the study period from January 2021 to July 2021—namely, ChAdOx1 (Oxford/AstraZeneca), BNT162b2 (BioNTech/Pfizer]) Ad26.COV2.S (Janssen) and mRNA-1273 (Moderna), were included for this study.

Post-COVID-19 outcomes of interest

Outcomes of interest were defined as SARS-CoV-2 infection followed by a predefined thromboembolic or cardiac event of interest within a year after infection, and with no record of the same clinical event in the 6 months before COVID-19. Outcome date was set as the corresponding SARS-CoV-2 infection date.

COVID-19 was identified from either a positive SARS-CoV-2 test (polymerase chain reaction (PCR) or antigen), or a clinical COVID-19 diagnosis, with no record of COVID-19 in the previous 6 weeks. This wash-out period was imposed to exclude re-recordings of the same COVID-19 episode.

Post-COVID-19 outcome events were selected based on previous studies.[11–13] Events comprised ischaemic stroke (IS), haemorrhagic stroke (HS), transient ischaemic attack (TIA), ventricular arrhythmia/cardiac arrest (VACA), myocarditis/pericarditis (MP), myocardial infarction (MI), heart failure (HF), pulmonary embolism (PE) and deep vein thrombosis (DVT). We used two composite outcomes: (1) VTE, as an aggregate of PE and DVT and (2) ATE, as a composite of IS, TIA and MI. To avoid re-recording of the same complication we imposed a wash-out period of 90 days between records. Phenotypes for these complications were based on previously published studies.[3 4 8 18]

All outcomes were ascertained in four different time periods following SARS-CoV-2 infection: the first period described the acute infection phase—that is, 0–30 days after COVID-19, whereas the later periods - which are 31–90 days, 91–180 days and 181–365 days, illustrate the post-acute phase (figure 1).

Negative control outcomes

Negative control outcomes (NCOs) were used to detect residual confounding. NCOs are outcomes which are not believed to be causally associated with the exposure, but share the same bias structure with the exposure and outcome of interest. Therefore, no significant association between exposure and NCO is to be expected. Our study used 43 different NCOs from previous work assessing vaccine effectiveness.[19]

Statistical analysis

Federated network analyses

A template for an analytical script was developed and subsequently tailored to include the country-specific aspects (eg, dates, priority groups) for the vaccination rollout. Analyses were conducted locally for each database. Only aggregated data were shared and person counts <5 were clouded.

Propensity score weighting

Large-scale propensity scores (PS) were calculated to estimate the likelihood of a person receiving the vaccine based on their demographic and health-related characteristics (eg, conditions,

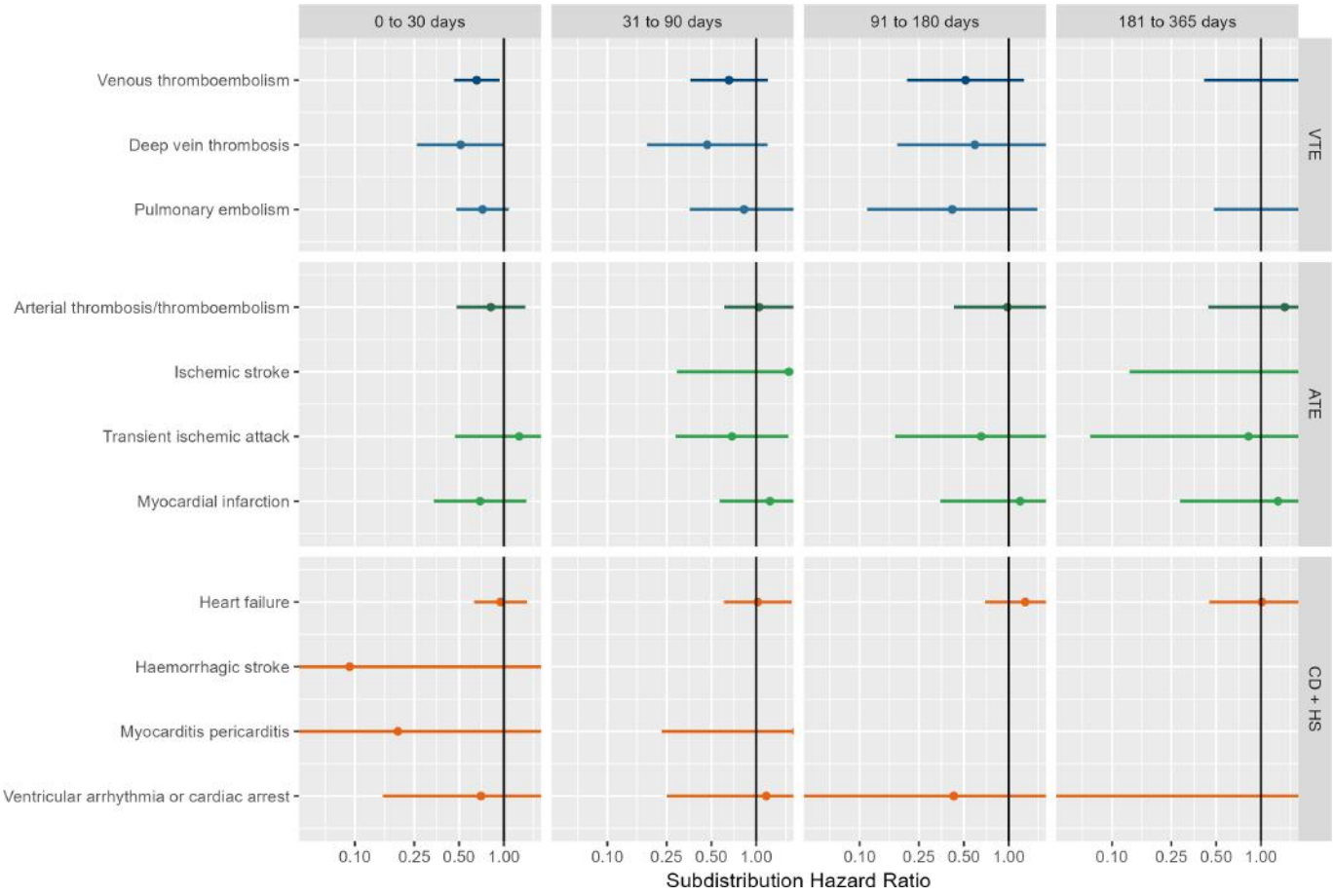

Figure 3 Forest plots for comparative vaccine effect (BNT162b2 vs ChAdOx1); meta-analysis across cohorts and databases. ATE, arterial thrombosis/thromboembolism; CD+HS, cardiac diseases and haemorrhagic stroke; VTE, venous thromboembolism.

medications) prior to the index date. PS were then used to minimise observed confounding by creating a weighted population (overlap weighting[20]), in which individuals contributed with a different weight based on their PS and vaccination status.

Prespecified key variables included in the PS comprised age, sex, location, index date, prior observation time in the database, number of previous outpatient visits and previous SARS-CoV-2 PCR/antigen tests. Regional vaccination, testing and COVID-19 incidence rates were also forced into the PS equation for the UK databases[21] and SIDIAP.[22] In addition, least absolute shrinkage and selection operator (LASSO) regression, a technique for variable selection, was used to identify additional variables from all recorded conditions and prescriptions within 0–30 days, 31–180 days and 181-any time (conditions only) before the index date that had a prevalence of >0.5% in the study population.

PS were then separately estimated for each staggered cohort and analysis. We considered covariate balance to be achieved if absolute standardised mean differences (ASMDs) were ≤0.1 after weighting. Baseline characteristics such as demographics and comorbidities were reported.

Effect estimation

To account for the competing risk of death associated with COVID-19, Fine-and-Grey models[23] were used to calculate subdistribution hazard ratios (sHRs). Subsequently, sHRs and confidence intervals were empirically calibrated from NCO estimates[24] to account for unmeasured confounding. To calibrate the estimates, the empirical null distribution was derived from NCO estimates and was used to compute calibrated confidence intervals. For each outcome, sHRs from the four staggered cohorts were pooled using random-effect meta-analysis, both separately for each database and across all four databases.

Sensitivity analysis

Sensitivity analyses comprised 1) censoring follow-up for vaccinated people at the time when they received their second vaccine dose and 2) considering only the first post-COVID-19 outcome within the year after infection (online supplemental figure S1). In addition, comparative effectiveness analyses were conducted for BNT162b2 versus ChAdOx1.

Data and code availability

All analytic code for the study is available in GitHub (https://github.com/oxford-pharmacoepi/vaccineEffectOnPostCovidCardiacThromboembolicEvents), including code lists for vaccines, COVID-19 tests and diagnoses, cardiac and thromboembolic events, NCO and health conditions to prioritise patients for vaccination in each country. We used R version 4.2.3 and statistical packages survival (3.5–3), Empirical Calibration (3.1.1), glmnet (4.1-7), and Hmisc (5.0–1).

Patient and public involvement

Owing to the nature of the study and the limitations regarding data privacy, the study design, analysis, interpretation of data

and revision of the manuscript did not involve any patients or members of the public.

RESULTS

All aggregated results are available in a web application (https://dpa-pde-oxford.shinyapps.io/PostCovidComplications/).

We included over 10.17 million vaccinated individuals (1 618 395 from CPRD Gold; 5 729 800 from CPRD Aurum; 2 744 821 from SIDIAP and 77 603 from CORIVA) and 10.39 million unvaccinated individuals (1 640 371; 5 860 564; 2 588 518 and 302 267, respectively). Online supplemental figures S2-5 illustrate study inclusion for each database.

Adequate covariate balance was achieved after PS weighting in most studies: CORIVA (all cohorts) and SIDIAP (cohorts 1 and 4) did not contribute to ChAdOx1 subanalyses owing to sample size and covariate imbalance. ASMD results are accessible in the web application.

NCO analyses suggested residual bias after PS weighting, with a majority of NCOs associated positively with vaccination. Therefore, calibrated estimates are reported in this manuscript. Uncalibrated effect estimates and NCO analyses are available in the web interface.

Population characteristics

Table 1 presents baseline characteristics for the weighted populations in CPRD Aurum, for illustrative purposes. Online supplemental tables S1-25 summarise baseline characteristics for weighted and unweighted populations for each database and comparison. Across databases and cohorts, populations followed similar patterns: cohort 1 represented an older subpopulation (around 80 years old) with a high proportion of women (57%). Median age was lowest in cohort 4 ranging between 30 and 40 years.

COVID-19 vaccination and post-COVID-19 complications

Table 2 shows the incidence of post-COVID-19 VTE, ATE and HF, the three most common post-COVID-19 conditions among the studied outcomes. Outcome counts are presented separately for 0–30, 31–90, 91–180 and 181–365 days after SARS-CoV-2 infection. Online supplemental tables S26-36 include all studied complications, also for the sensitivity and subanalyses. Similar pattern for incidences were observed across all databases: higher outcome rates in the older populations (cohort 1) and decreasing frequency with increasing time after infection in all cohorts.

Results from calibrated estimates pooled in meta-analysis across cohorts and databases are shown in figure 2.

Reduced risk associated with vaccination is observed for acute and post-acute VTE, DVT, and PE: acute meta-analytic sHR are 0.22 (95% CI, 0.17–0.29); 0.36 (0.28–0.45); and 0.19 (0.15–0.25), respectively. For VTE in the post-acute phase, sHR estimates are 0.43 (0.34–0.53), 0.53 (0.40–0.70) and 0.50 (0.36–0.70) for 31–90, 91–180, and 181–365 days post COVID-19, respectively. Reduced risk of VTE outcomes was observed in vaccinated across databases and cohorts, see online supplemental figures S14–22.

Similarly, the risk of ATE, IS and MI in the acute phase after infection was reduced for the vaccinated group, sHR of 0.53 (0.44–0.63), 0.55 (0.43–0.70) and 0.49 (0.38–0.62), respectively. Reduced risk associated with vaccination persisted for post-acute ATE, with sHR of 0.74 (0.60–0.92), 0.72 (0.58–0.88) and 0.62 (0.48–0.80) for 31–90, 91–180 and 181–365 days post-COVID-19, respectively. Risk of post-acute MI remained lower for vaccinated in the 31–90 and 91–180 days after COVID-19, with sHR of 0.64 (0.46–0.87) and 0.64 (0.45–0.90), respectively. Vaccination effect on post-COVID-19 TIA was seen only in the 181–365 days, with sHR of 0.51 (0.31–0.82). Online supplemental figures S23-31 show database-specific and cohort-specific estimates for ATE-related complications.

Risk of post-COVID-19 cardiac complications was reduced in vaccinated individuals. Meta-analytic estimates in the acute phase showed sHR of 0.45 (0.38–0.53) for HF, 0.41 (0.26–0.66) for MP and 0.41 (0.27–0.63) for VACA. Reduced risk persisted for post-acute COVID-19 HF: sHR 0.61 (0.51–0.73) for 31–90 days, 0.61 (0.51–0.73) for 91–180 days and 0.52 (0.43–0.63) for 181–365 days. For post-acute MP, risk was only lowered in the first post-acute window (31–90 days), with sHR of 0.43 (0.21–0.85). Vaccination showed no association with post-COVID-19 HS. Database-specific and cohort-specific results for these cardiac diseases are shown in online supplemental figures S32-40.

Stratified analyses by vaccine showed similar associations, except for ChAdOx1 which was not associated with reduced VTE and ATE risk in the last post-acute window. Sensitivity analyses were consistent with main results (online supplemental figures S6-13).

Figure 3 shows the results of comparative effects of BNT162b2 versus ChAdOx1, based on UK data. Meta-analytic estimates favoured BNT162b2 (sHR of 0.66 (0.46–0.93)) for VTE in the 0–30 days after infection, but no differences were seen for post-acute VTE or for any of the other outcomes. Results from sensitivity analyses, database-specific and cohort-specific estimates were in line with the main findings (online supplemental figures S41-51).

DISCUSSION

Key findings

Our analyses showed a substantial reduction of risk (45–81%) for thromboembolic and cardiac events in the acute phase of COVID-19 associated with vaccination. This finding was consistent across four databases and three different European countries. Risks for post-acute COVID-19 VTE, ATE and HF were reduced to a lesser extent (24–58%), whereas a reduced risk for post-COVID-19 MP and VACA in vaccinated people was seen only in the acute phase.

Results in context

The relationship between SARS-CoV-2 infection, COVID-19 vaccines and thromboembolic and/or cardiac complications is tangled. Some large studies report an increased risk of VTE and ATE following both ChAdOx1 and BNT162b2 vaccination,[7] whereas other studies have not identified such a risk.[25] Elevated risk of VTE has also been reported among patients with COVID-19 and its occurrence can lead to poor prognosis and mortality.[26 27] Similarly, several observational studies have found an association between COVID-19 mRNA vaccination and a short-term increased risk of myocarditis, particularly among younger male individuals.[5 6] For instance, a self-controlled case series study conducted in England revealed about 30% increased risk of hospital admission due to myocarditis within 28 days following both ChAdOx1 and BNT162b2 vaccines. However, this same study also found a ninefold higher risk for myocarditis following a positive SARS-CoV-2 test, clearly offsetting the observed post-vaccine risk.

COVID-19 vaccines have demonstrated high efficacy and effectiveness in preventing infection and reducing the severity of acute-phase infection. However, with the emergence of

newer variants of the virus, such as omicron, and the waning protective effect of the vaccine over time, there is a growing interest in understanding whether the vaccine can also reduce the risk of complications after breakthrough infections. Recent studies suggested that COVID-19 vaccination could potentially protect against acute post-COVID-19 cardiac and thromboembolic events.[11 12] A large prospective cohort study[11] reports risk of VTE after SARS-CoV-2 infection to be substantially reduced in fully vaccinated ambulatory patients. Likewise, Al-Aly *et al*[12] suggest a reduced risk for post-acute COVID-19 conditions in breakthrough infection versus SARS-CoV-2 infection without prior vaccination. However, the populations were limited to SARS-CoV-2 infected individuals and estimates did not include the effect of the vaccine to prevent COVID-19 in the first place. Other studies on post-acute COVID-19 conditions and symptoms have been conducted,[28 29] but there has been limited reporting on the condition-specific risks associated with COVID-19, even though the prognosis for different complications can vary significantly.

In line with previous studies, our findings suggest a potential benefit of vaccination in reducing the risk of post-COVID-19 thromboembolic and cardiac complications. We included broader populations, estimated the risk in both acute and post-acute infection phases and replicated these using four large independent observational databases. By pooling results across different settings, we provided the most up-to-date and robust evidence on this topic.

Strengths and limitations

The study has several strengths. Our multinational study covering different healthcare systems and settings showed consistent results across all databases, which highlights the robustness and replicability of our findings. All databases had complete recordings of vaccination status (date and vaccine) and are representative of the respective general population. Algorithms to identify study outcomes were used in previous published network studies, including regulatory-funded research.[3 4 8 18] Other strengths are the staggered cohort design which minimises confounding by indication and immortal time bias. PS overlap weighting and NCO empirical calibration have been shown to adequately minimise bias in vaccine effectiveness studies.[19] Furthermore, our estimates include the vaccine effectiveness against COVID-19, which is crucial in the pathway to experience post-COVID-19 complications.

Our study has some limitations. The use of real-world data comes with inherent limitations including data quality concerns and risk of confounding. To deal with these limitations, we employed state-of-the-art methods, including large-scale propensity score weighting and calibration of effect estimates using NCO.[19 24] A recent study[30] has demonstrated that methodologically sound observational studies based on routinely collected data can produce results similar to those of clinical trials. We acknowledge that results from NCO were positively associated with vaccination, and estimates might still be influenced by residual bias despite using calibration. Another limitation is potential under-reporting of post-COVID-19 complications: some asymptomatic and mild COVID-19 infections might have not been recorded. Additionally, post-COVID-19 outcomes of interest might be under-recorded in primary care databases (CPRD Aurum and Gold) without hospital linkage, which represent a large proportion of the data in the study. However, results in SIDIAP and CORIVA, which include secondary care data, were similar. Also, our study included a small number of young men and male teenagers, who were the main population concerned with increased risks of myocarditis/pericarditis following vaccination.

CONCLUSIONS

Vaccination against SARS-CoV-2 substantially reduced the risk of acute post-COVID-19 thromboembolic and cardiac complications, probably through a reduction in the risk of SARS-CoV-2 infection and the severity of COVID-19 disease due to vaccine-induced immunity. Reduced risk in vaccinated people lasted for up to 1 year for post-COVID-19 VTE, ATE and HF, but not clearly for other complications. Findings from this study highlight yet another benefit of COVID-19 vaccination. However, further research is needed on the possible waning of the risk reduction over time and on the impact of booster vaccination.

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Contributors DPA and AMJ led the conceptualisation of the study with contributions from MC and NM-B. AMJ, TD-S, ER, AU and NTHT adapted the study design with respect to the local vaccine rollouts. AD and WYM mapped and curated CPRD data. MC and NM-B developed code with methodological contributions advice from MTS-S and CP. DPA, MC, NTHT, TD-S, HMEN, XL, CR and AMJ clinically interpreted the results. NM-B, XL, AMJ and DPA wrote the first draft of the manuscript, and all authors read, revised and approved the final version. DPA and AMJ obtained the funding for this research. DPA is responsible for the overall content as guarantor: he accepts full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

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Data availability statement Data may be obtained from a third party and are not publicly available. CPRD: CPRD data were obtained under the CPRD multi-study license held by the University of Oxford after Research Data Governance (RDG) approval. Direct data sharing is not allowed. SIDIAP: In accordance with current European and national law, the data used in this study is only available for the researchers participating in this study. Thus, we are not allowed to distribute or make publicly available the data to other parties. However, researchers from public institutions can request data from SIDIAP if they comply with certain requirements. Further information is available online (https://www.sidiap.org/index.php/menu-solicitudesen/application-proccedure) or by contacting SIDIAP (sidiap@idiapjgol.org). CORIVA: CORIVA data were obtained under the approval of Research Ethics Committee of the University of Tartu and the patient level data sharing is not allowed. All analyses in this study were conducted in a federated manner, where analytical code and aggregated (anonymised) results were shared, but no patient-level data was transferred across the collaborating institutions.

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SUPPLEMENT

The effectiveness of COVID-19 vaccines to prevent post COVID cardiac and thromboembolic complications.

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(A) Main analysis, (B) Follow-up ends at first vaccine dose after index date, (C) Post COVID sequelae refers just to the first outcome after infection.

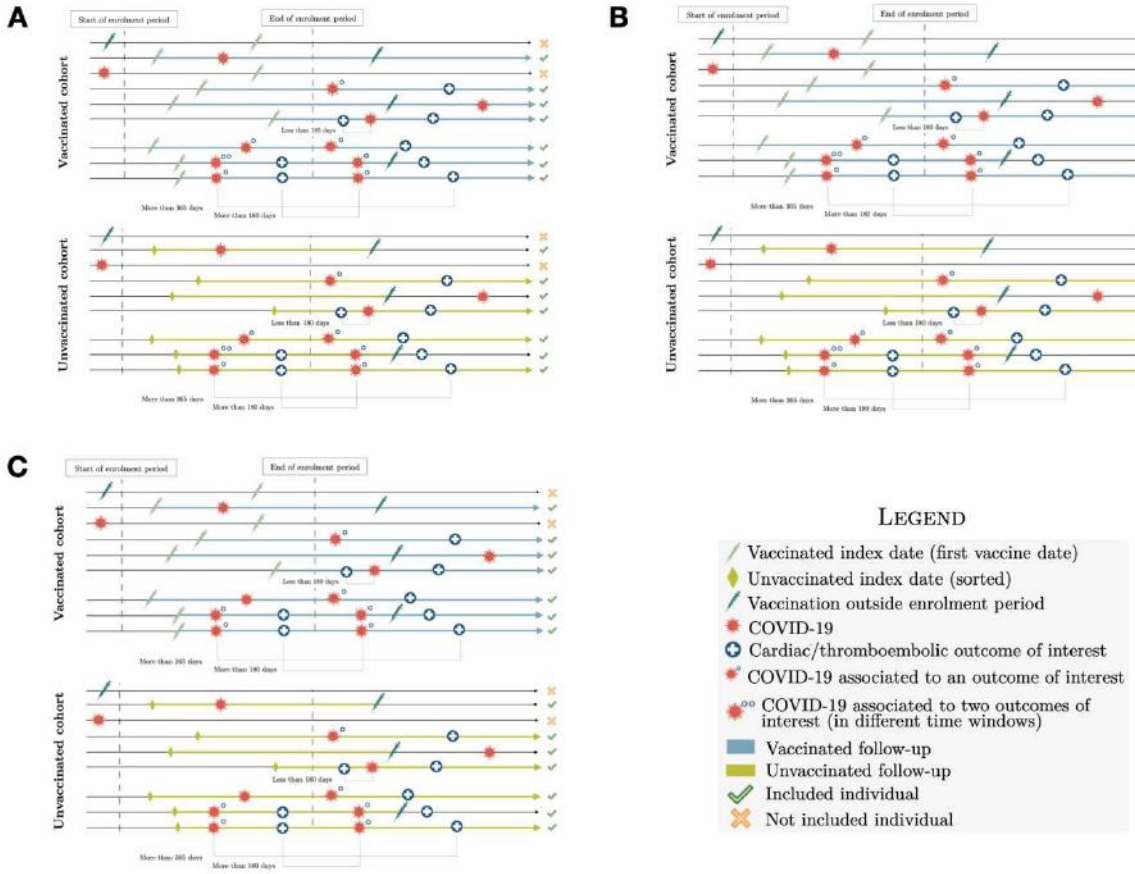


Figure S2: Study Inclusion Flowchart CPRD AURUM

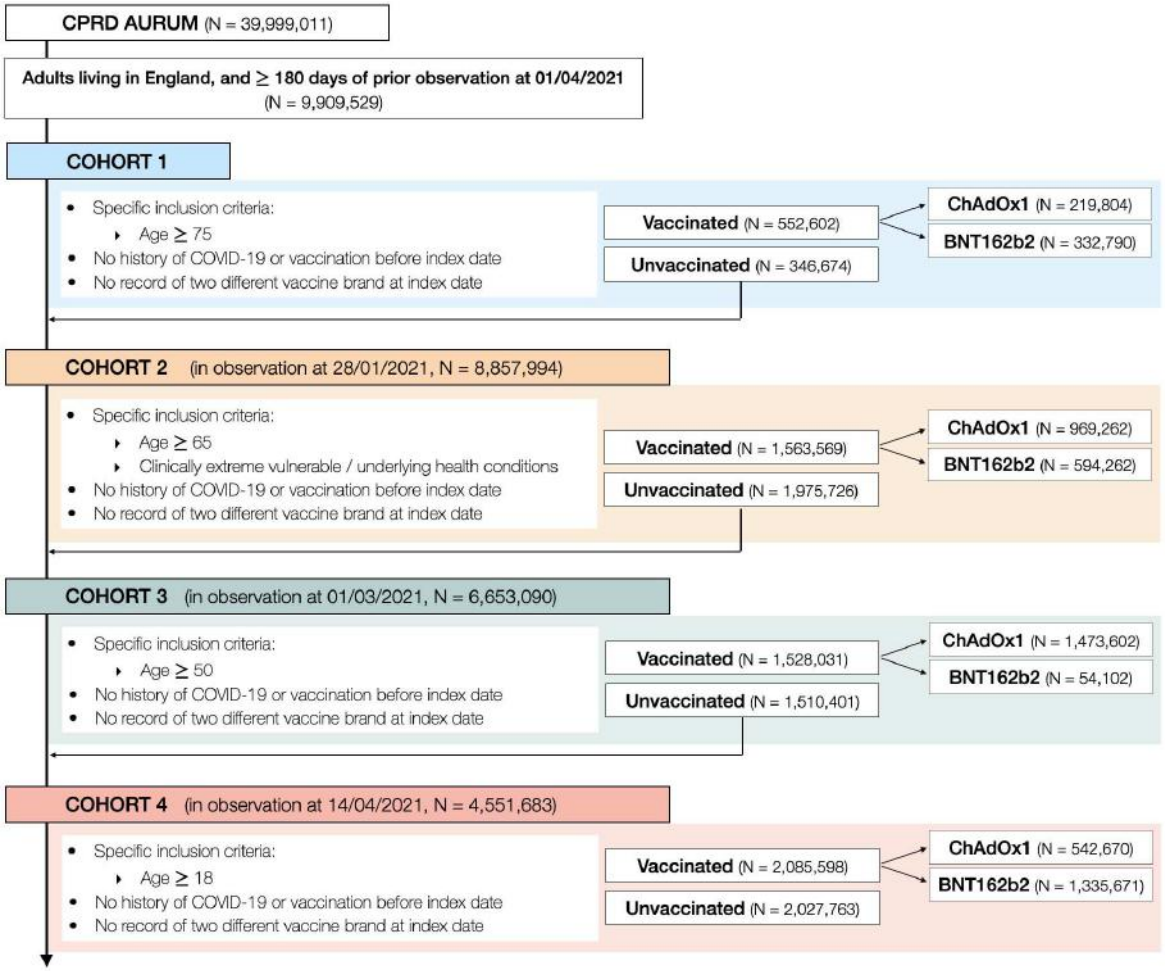


Figure S3: Study Inclusion Flowchart CPRD GOLD.

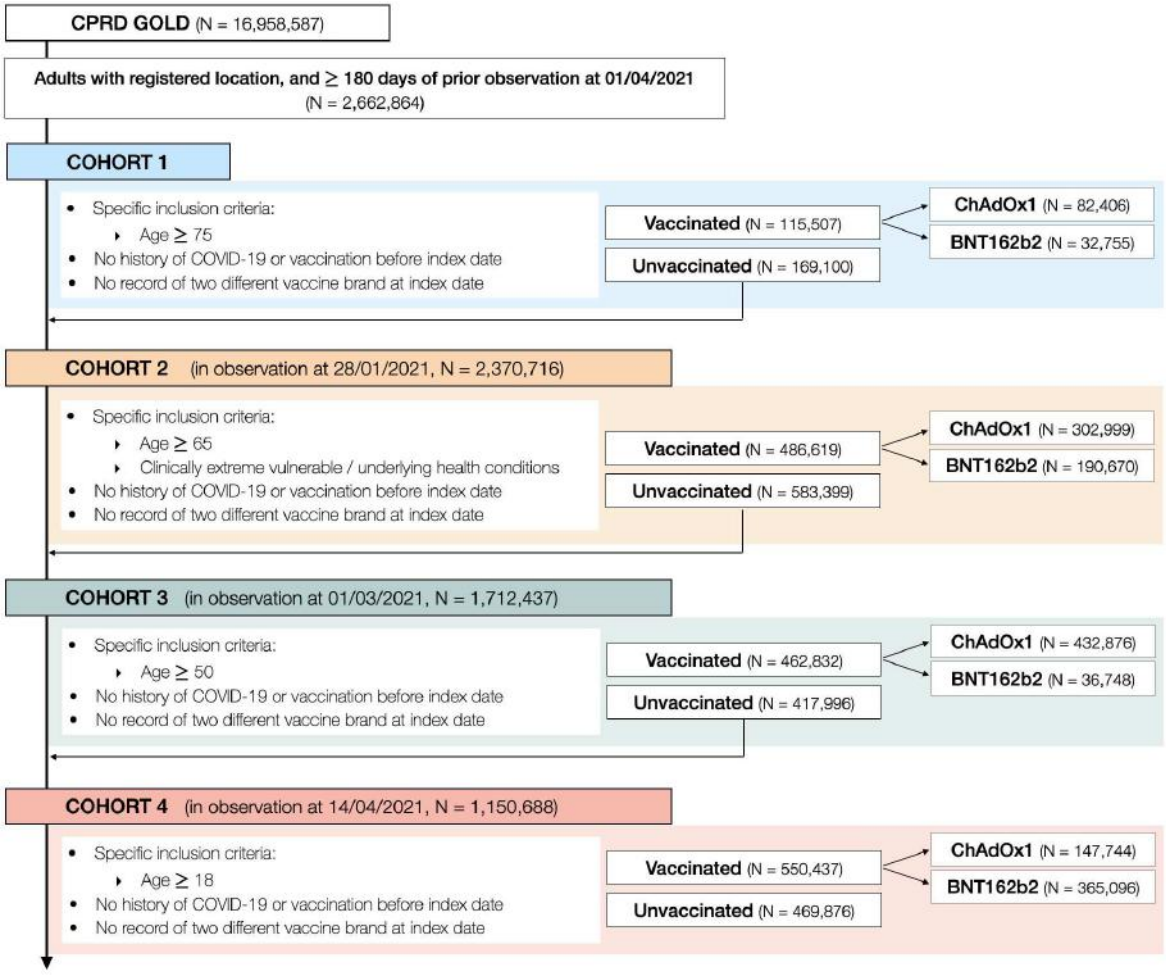


Figure S4: Study Inclusion Flowchart SIDIAP.

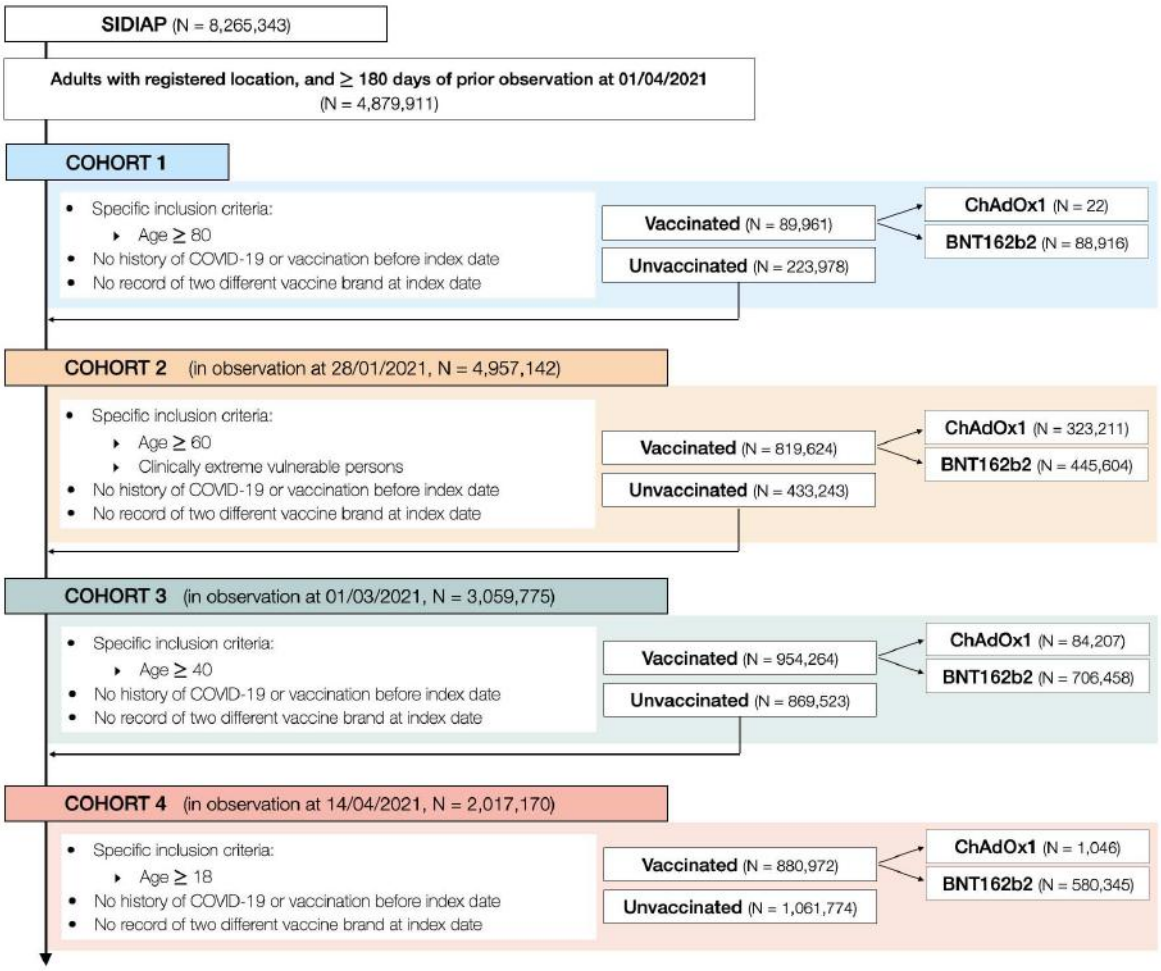


Figure S5: Study Inclusion Flowchart CORIVA.

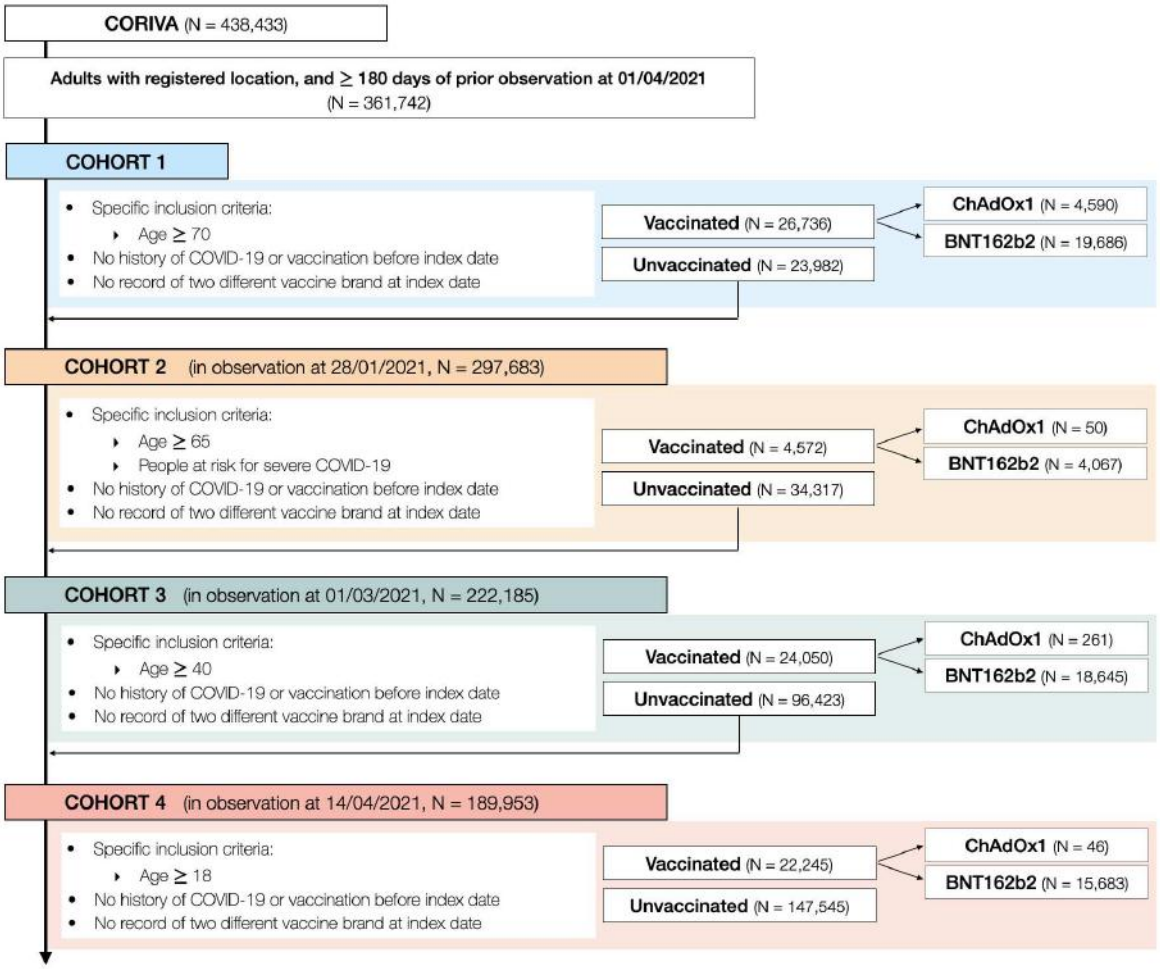


Table S1: Characteristics of unweighted populations in CPRD AURUM, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	346,674	552,602		1,975,726	1,563,569		1,510,401	1,528,031		2,027,763	2,085,598	
Age, median [Q25-Q75]	78 [76-82]	81 [78-86]	0.319	42 [32-55]	68 [60-73]	0.995	41 [31-53]	55 [51-60]	0.610	35 [27-45]	35 [27-43]	0.134
Sex: Female, N(%)	192,771 (56%)	314,141 (57%)	0.025	1,244,636 (63%)	852,976 (55%)	0.172	880,404 (58%)	771,929 (51%)	0.157	862,676 (43%)	958,124 (46%)	0.068
Years of prior history*, median [Q25-Q75]	24 [10-35]	25 [11-37]	0.056	15 [7-25]	21 [9-32]	0.278	13 [6-22]	17 [8-27]	0.207	8 [4-16]	8 [3-18]	0.032
Number of GP visits, median [Q25-Q75]	10 [5-17]	11 [6-19]	0.105	6 [2-11]	10 [6-17]	0.367	4 [1-10]	7 [3-12]	0.180	2 [0-5]	3 [1-7]	0.108
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.103	0[0-0]	0[0-0]	0.065	0[0-0]	0[0-0]	0.007	0[0-0]	0[0-0]	0.078
Comorbidities, N(%)**												
Anxiety	51,067 (15%)	82,765 (15%)	0.007	435,770 (22%)	309,567 (20%)	0.056	289,563 (19%)	292,336 (19%)	0.001	265,627 (13%)	338,058 (16%)	0.088
Asthma	36,474 (11%)	61,899 (11%)	0.022	501,209 (25%)	275,768 (18%)	0.189	294,123 (19%)	209,708 (14%)	0.155	138,317 (7%)	173,056 (8%)	0.056
Chronic kidney disease	71,083 (21%)	137,953 (25%)	0.107	55,104 (3%)	154,263 (10%)	0.294	24,340 (2%)	30,896 (2%)	0.031	18,625 (1%)	5,796 (0%)	0.083
COPD	28,665 (8%)	47,422 (9%)	0.011	29,729 (2%)	107,297 (7%)	0.270	13,433 (1%)	15,685 (1%)	0.014	10,543 (1%)	2,535 (0%)	0.071
Dementia	16,610 (5%)	32,934 (6%)	0.052	6,692 (0%)	15,828 (1%)	0.082	3,425 (0%)	3,219 (0%)	0.003	2,691 (0%)	575 (0%)	0.037
Depressive disorder	42,145 (12%)	64,840 (12%)	0.013	367,463 (19%)	283,390 (18%)	0.012	236,584 (16%)	265,876 (17%)	0.047	210,644 (10%)	253,317 (12%)	0.056
Diabetes	63,154 (18%)	101,682 (18%)	0.005	107,425 (5%)	253,026 (16%)	0.351	60,932 (4%)	84,224 (6%)	0.069	49,154 (2%)	22,968 (1%)	0.101
GERD	18,336 (5%)	32,360 (6%)	0.025	68,070 (3%)	84,985 (5%)	0.097	39,594 (3%)	58,961 (4%)	0.070	31,119 (2%)	38,612 (2%)	0.025
Heart failure	17,882 (5%)	33,578 (6%)	0.040	12,275 (1%)	37,396 (2%)	0.146	5,641 (0%)	7,204 (0%)	0.015	4,611 (0%)	1,163 (0%)	0.046
Hypertension	172,799 (50%)	301,294 (55%)	0.094	206,105 (10%)	545,782 (35%)	0.611	110,912 (7%)	240,120 (16%)	0.264	86,435 (4%)	54,357 (3%)	0.091
Hypothyroidism	31,444 (9%)	56,367 (10%)	0.038	78,934 (4%)	118,453 (8%)	0.154	43,892 (3%)	67,686 (4%)	0.081	30,993 (2%)	35,364 (2%)	0.013
Malignant neoplastic	68,984 (20%)	131,635	0.095	60,318 (3%)	207,240	0.379	29,689 (2%)	61,923 (4%)	0.122	22,701 (1%)	13,590 (1%)	0.050

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
disease		(24%)			(13%)							
Myocardial infarction	16,027 (5%)	29,525 (5%)	0.033	20,259 (1%)	56,161 (4%)	0.172	7,396 (0%)	12,703 (1%)	0.042	5,764 (0%)	1,621 (0%)	0.049
Osteoporosis	29,909 (9%)	59,521 (11%)	0.072	18,263 (1%)	64,108 (4%)	0.204	8,846 (1%)	15,089 (1%)	0.045	6,922 (0%)	2,535 (0%)	0.046
Pneumonia	16,992 (5%)	28,398 (5%)	0.011	31,746 (2%)	50,739 (3%)	0.107	17,785 (1%)	22,064 (1%)	0.023	13,305 (1%)	13,282 (1%)	0.002
Rheumatoid arthritis	6,596 (2%)	11,237 (2%)	0.009	14,811 (1%)	33,173 (2%)	0.116	5,548 (0%)	9,130 (1%)	0.033	3,710 (0%)	1,816 (0%)	0.026
Stroke	15,249 (4%)	26,478 (5%)	0.019	16,942 (1%)	39,907 (3%)	0.131	7,422 (0%)	10,828 (1%)	0.028	6,030 (0%)	2,291 (0%)	0.042
Venous thromboembolism	19,607 (6%)	35,332 (6%)	0.031	28,876 (1%)	63,048 (4%)	0.158	13,608 (1%)	26,299 (2%)	0.072	12,945 (1%)	4,787 (0%)	0.062

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S2: Characteristics of weighted populations in CPRD AURUM, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	100,517	99,985		308,608	304,995		448,766	445,397		242,955	243,847	
Age, median [Q25-Q75]	78 [76-84]	78 [76-84]	0.001	62 [47-69]	62 [47-69]	0.007	50 [41-57]	52 [41-57]	0.001	44 [41-48]	44 [41-48]	0.003
Sex: Female, N(%)	57,756 (57%)	57,174 (57%)	0.006	179,574 (58%)	176,871 (58%)	0.004	236,260 (53%)	234,320 (53%)	0.001	101,805 (42%)	100,846 (41%)	0.011
Years of prior history*, median [Q25-Q75]	24 [10-36]	24 [10-36]	0.004	18 [8-29]	18 [8-29]	0.002	14 [6-24]	14 [6-24]	0.002	10 [5-18]	9 [5-18]	0.004
Number of GP visits, median [Q25-Q75]	10 [5-18]	10 [6-18]	0.003	8 [3-15]	8 [5-15]	0.003	4 [1-11]	6 [2-10]	0.007	2 [0-7]	2 [1-7]	0.016
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.011	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.005
Comorbidities, N(%)**												
Anxiety	15,457 (15%)	15,069 (15%)	0.009	67,125 (22%)	64,408 (21%)	0.015	90,806 (20%)	87,243 (20%)	0.016	37,475 (15%)	38,040 (16%)	0.005
Asthma	10,993 (11%)	10,863 (11%)	0.002	65,274 (21%)	62,424 (20%)	0.017	76,417 (17%)	75,841 (17%)	0.000	17,944 (7%)	17,468 (7%)	0.009
Chronic kidney disease	23,223 (23%)	22,872 (23%)	0.005	23,022 (7%)	24,288 (8%)	0.019	8,460 (2%)	8,356 (2%)	0.001	2,419 (1%)	2,043 (1%)	0.017
COPD	8,669 (9%)	8,770 (9%)	0.005	14,411 (5%)	15,226 (5%)	0.015	5,747 (1%)	4,794 (1%)	0.019	1,263 (1%)	1,250 (1%)	0.001
Dementia	6,529 (6%)	6,431 (6%)	0.003	3,880 (1%)	3,541 (1%)	0.009	898 (0%)	1,004 (0%)	0.006	203 (0%)	399 (0%)	0.023
Depressive disorder	12,559 (12%)	12,267 (12%)	0.007	60,745 (20%)	57,903 (19%)	0.018	80,264 (18%)	77,017 (17%)	0.016	32,304 (13%)	33,302 (14%)	0.011
Diabetes	18,750 (19%)	18,602 (19%)	0.001	39,262 (13%)	38,979 (13%)	0.002	25,334 (6%)	26,121 (6%)	0.009	6,679 (3%)	6,315 (3%)	0.010
GERD	5,590 (6%)	5,471 (5%)	0.004	14,645 (5%)	14,339 (5%)	0.002	14,758 (3%)	14,823 (3%)	0.002	5,727 (2%)	6,021 (2%)	0.007
Heart failure	6,181 (6%)	5,763 (6%)	0.016	6,098 (2%)	5,685 (2%)	0.008	2,241 (0%)	1,983 (0%)	0.008	610 (0%)	545 (0%)	0.006
Hypertension	52,737 (52%)	51,796 (52%)	0.013	78,623 (25%)	79,827 (26%)	0.016	51,054 (11%)	51,059 (11%)	0.003	15,350 (6%)	15,670 (6%)	0.004
Hypothyroidism	9,956 (10%)	9,690 (10%)	0.007	19,720 (6%)	19,562 (6%)	0.001	16,480 (4%)	16,361 (4%)	0.000	5,735 (2%)	5,984 (2%)	0.006
Malignant neoplastic	21,691 (22%)	21,468	0.003	25,191 (8%)	27,892 (9%)	0.035	13,140 (3%)	12,870 (3%)	0.002	4,003 (2%)	3,621 (1%)	0.013

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
disease		(21%)										
Myocardial infarction	5,182 (5%)	4,988 (5%)	0.008	7,777 (3%)	8,594 (3%)	0.018	3,415 (1%)	3,228 (1%)	0.004	933 (0%)	636 (0%)	0.022
Osteoporosis	9,975 (10%)	10,018 (10%)	0.003	9,052 (3%)	9,423 (3%)	0.009	3,285 (1%)	3,499 (1%)	0.006	840 (0%)	934 (0%)	0.006
Pneumonia	5,770 (6%)	5,268 (5%)	0.021	9,096 (3%)	8,541 (3%)	0.009	6,196 (1%)	6,077 (1%)	0.001	1,923 (1%)	2,040 (1%)	0.005
Rheumatoid arthritis	2,002 (2%)	2,063 (2%)	0.005	4,856 (2%)	4,971 (2%)	0.004	2,266 (1%)	2,795 (1%)	0.016	665 (0%)	478 (0%)	0.016
Stroke	5,085 (5%)	4,791 (5%)	0.012	6,650 (2%)	6,616 (2%)	0.001	3,150 (1%)	2,942 (1%)	0.005	908 (0%)	773 (0%)	0.010
Venous thromboembolism	6,342 (6%)	6,069 (6%)	0.010	9,208 (3%)	9,850 (3%)	0.014	6,193 (1%)	7,393 (2%)	0.023	2,532 (1%)	1,239 (1%)	0.061

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S3: Characteristics of unweighted populations in CPRD AURUM, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	344,687	219,804		1,975,770	969,262		1,510,493	1,473,602		2,066,318	542,670	
Age, median [Q25-Q75]	78 [76-82]	80 [77-85]	0.240	42 [32-55]	69 [63-73]	1.059	41 [31-53]	55 [51-60]	0.605	34 [27-45]	45 [42-48]	0.507
Sex: Female, N(%)	191,649 (56%)	127,656 (58%)	0.050	1,244,532 (63%)	528,692 (55%)	0.172	880,468 (58%)	739,444 (50%)	0.163	880,225 (43%)	242,758 (45%)	0.043
Years of prior history*, median [Q25-Q75]	24 [10-35]	24 [9-36]	0.018	15 [7-25]	21 [9-33]	0.288	13 [6-22]	17 [8-27]	0.203	7 [4-16]	10 [5-18]	0.143
Number of GP visits, median [Q25-Q75]	10 [5-17]	12 [6-19]	0.121	6 [2-11]	10 [6-17]	0.359	4 [1-10]	7 [3-12]	0.169	2 [0-5]	3 [1-8]	0.146
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.194	0[0-0]	0[0-0]	0.070	0[0-0]	0[0-0]	0.005	0[0-0]	0[0-0]	0.066
Comorbidities, N(%)**												
Anxiety	50,786 (15%)	33,355 (15%)	0.012	435,737 (22%)	187,469 (19%)	0.067	289,618 (19%)	281,846 (19%)	0.001	268,451 (13%)	86,805 (16%)	0.085
Asthma	36,259 (11%)	24,009 (11%)	0.013	501,234 (25%)	159,162 (16%)	0.221	294,156 (19%)	200,995 (14%)	0.157	140,178 (7%)	41,393 (8%)	0.033
Chronic kidney disease	70,604 (20%)	51,053 (23%)	0.066	55,103 (3%)	97,339 (10%)	0.299	24,361 (2%)	24,198 (2%)	0.002	18,798 (1%)	2,879 (1%)	0.045
COPD	28,448 (8%)	19,105 (9%)	0.016	29,730 (2%)	67,700 (7%)	0.274	13,436 (1%)	13,621 (1%)	0.004	10,647 (1%)	1,510 (0%)	0.038
Dementia	16,327 (5%)	16,795 (8%)	0.121	6,689 (0%)	12,106 (1%)	0.103	3,424 (0%)	1,896 (0%)	0.023	2,729 (0%)	447 (0%)	0.015
Depressive disorder	41,905 (12%)	27,009 (12%)	0.004	367,500 (19%)	170,626 (18%)	0.026	236,628 (16%)	256,789 (17%)	0.047	212,626 (10%)	74,210 (14%)	0.104
Diabetes	62,733 (18%)	39,751 (18%)	0.003	107,449 (5%)	154,348 (16%)	0.344	60,968 (4%)	78,394 (5%)	0.061	49,223 (2%)	9,729 (2%)	0.041
GERD	18,253 (5%)	12,156 (6%)	0.010	68,089 (3%)	52,694 (5%)	0.097	39,619 (3%)	56,444 (4%)	0.068	31,367 (2%)	13,807 (3%)	0.073
Heart failure	17,716 (5%)	13,147 (6%)	0.037	12,277 (1%)	24,276 (3%)	0.152	5,638 (0%)	5,673 (0%)	0.002	4,625 (0%)	681 (0%)	0.024
Hypertension	171,846 (50%)	114,922 (52%)	0.049	206,105 (10%)	345,014 (36%)	0.626	110,975 (7%)	224,543 (15%)	0.251	87,010 (4%)	31,330 (6%)	0.072
Hypothyroidism	31,256 (9%)	21,855 (10%)	0.030	78,921 (4%)	73,992 (8%)	0.156	43,887 (3%)	64,026 (4%)	0.077	31,315 (2%)	13,920 (3%)	0.074
Malignant neoplastic disease	68,598 (20%)	49,023 (22%)	0.059	60,355 (3%)	131,135 (14%)	0.387	29,693 (2%)	55,601 (4%)	0.108	22,873 (1%)	7,505 (1%)	0.025

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	15,938 (5%)	11,236 (5%)	0.023	20,262 (1%)	33,142 (3%)	0.163	7,401 (0%)	11,237 (1%)	0.035	5,781 (0%)	861 (0%)	0.026
Osteoporosis	29,704 (9%)	22,986 (10%)	0.063	18,255 (1%)	42,319 (4%)	0.216	8,856 (1%)	12,455 (1%)	0.031	6,980 (0%)	1,380 (0%)	0.015
Pneumonia	16,837 (5%)	12,101 (6%)	0.028	31,706 (2%)	33,170 (3%)	0.116	17,787 (1%)	20,399 (1%)	0.018	13,425 (1%)	4,297 (1%)	0.017
Rheumatoid arthritis	6,549 (2%)	4,544 (2%)	0.012	14,813 (1%)	20,294 (2%)	0.114	5,536 (0%)	8,463 (1%)	0.030	3,723 (0%)	821 (0%)	0.007
Stroke	15,118 (4%)	10,876 (5%)	0.027	16,944 (1%)	24,851 (3%)	0.132	7,424 (0%)	9,547 (1%)	0.021	6,059 (0%)	1,153 (0%)	0.016
Venous thromboembolism	19,455 (6%)	13,782 (6%)	0.026	28,887 (1%)	39,415 (4%)	0.159	13,609 (1%)	24,312 (2%)	0.067	13,021 (1%)	1,936 (0%)	0.039

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S4: Characteristics of weighted populations in CPRD AURUM, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	105,853	106,684		255,400	253,164		37,236	37,227		630,449	633,628	
Age, median [Q25-Q75]	80 [77-85]	80 [77-85]	0.003	58 [46-67]	58 [46-68]	0.006	54 [45-64]	54 [45-64]	0.016	32 [25-37]	32 [25-37]	0.001
Sex: Female, N(%)	60,612 (57%)	60,891 (57%)	0.004	147,020 (58%)	146,572 (58%)	0.007	22,049 (59%)	22,144 (59%)	0.005	278,649 (44%)	282,744 (45%)	0.009
Years of prior history*, median [Q25-Q75]	24 [11-36]	24 [11-35]	0.005	18 [8-29]	18 [8-29]	0.007	16 [7-27]	17 [7-27]	0.007	7 [4-16]	7 [3-18]	0.006
Number of GP visits, median [Q25-Q75]	10 [5-18]	10 [6-17]	0.002	8 [3-15]	10 [5-15]	0.013	6 [2-15]	8 [4-14]	0.003	2 [0-6]	2 [0-6]	0.011
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.001	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.000	0[0-0]	0[0-0]	0.010
Comorbidities, N(%)**												
Anxiety	15,706 (15%)	15,578 (15%)	0.007	57,951 (23%)	55,594 (22%)	0.018	7,672 (21%)	7,363 (20%)	0.021	96,918 (15%)	96,528 (15%)	0.004
Asthma	11,563 (11%)	11,733 (11%)	0.002	60,265 (24%)	58,010 (23%)	0.016	6,397 (17%)	6,215 (17%)	0.013	50,849 (8%)	48,590 (8%)	0.015
Chronic kidney disease	25,741 (24%)	26,243 (25%)	0.007	17,219 (7%)	18,864 (7%)	0.028	2,790 (7%)	2,621 (7%)	0.017	2,035 (0%)	1,846 (0%)	0.006
COPD	9,240 (9%)	8,999 (8%)	0.011	11,625 (5%)	12,410 (5%)	0.017	1,224 (3%)	886 (2%)	0.055	859 (0%)	807 (0%)	0.002
Dementia	6,683 (6%)	5,727 (5%)	0.040	2,225 (1%)	1,309 (1%)	0.043	618 (2%)	529 (1%)	0.019	112 (0%)	107 (0%)	0.001
Depressive disorder	12,529 (12%)	12,450 (12%)	0.005	52,883 (21%)	50,765 (20%)	0.016	6,588 (18%)	6,431 (17%)	0.011	71,832 (11%)	71,139 (11%)	0.005
Diabetes	20,227 (19%)	20,182 (19%)	0.005	32,557 (13%)	32,989 (13%)	0.008	3,153 (8%)	3,212 (9%)	0.006	8,183 (1%)	7,675 (1%)	0.008
GERD	6,148 (6%)	5,937 (6%)	0.011	12,436 (5%)	12,160 (5%)	0.003	1,552 (4%)	1,509 (4%)	0.006	9,586 (2%)	10,173 (2%)	0.007
Heart failure	6,643 (6%)	6,329 (6%)	0.014	4,618 (2%)	3,987 (2%)	0.018	767 (2%)	552 (1%)	0.044	474 (0%)	344 (0%)	0.008
Hypertension	56,732 (54%)	57,023 (53%)	0.003	62,628 (25%)	63,500 (25%)	0.013	7,802 (21%)	7,697 (21%)	0.007	11,603 (2%)	12,098 (2%)	0.005
Hypothyroidism	10,528 (10%)	10,633 (10%)	0.001	16,084 (6%)	16,368 (6%)	0.007	2,046 (5%)	2,032 (5%)	0.002	8,722 (1%)	8,355 (1%)	0.006
Malignant neoplastic disease	23,367 (22%)	23,618 (22%)	0.002	19,432 (8%)	22,945 (9%)	0.053	2,799 (8%)	2,742 (7%)	0.006	3,432 (1%)	2,913 (0%)	0.012

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	5,451 (5%)	5,473 (5%)	0.001	6,811 (3%)	7,856 (3%)	0.026	704 (2%)	637 (2%)	0.013	607 (0%)	569 (0%)	0.002
Osteoporosis	10,923 (10%)	10,997 (10%)	0.000	6,664 (3%)	6,208 (2%)	0.010	1,166 (3%)	1,014 (3%)	0.024	653 (0%)	702 (0%)	0.002
Pneumonia	5,814 (5%)	5,303 (5%)	0.023	7,059 (3%)	6,337 (3%)	0.016	991 (3%)	831 (2%)	0.028	3,698 (1%)	3,579 (1%)	0.003
Rheumatoid arthritis	2,115 (2%)	2,076 (2%)	0.004	4,061 (2%)	4,486 (2%)	0.014	330 (1%)	382 (1%)	0.014	723 (0%)	523 (0%)	0.010
Stroke	5,304 (5%)	4,911 (5%)	0.019	5,139 (2%)	5,592 (2%)	0.014	657 (2%)	560 (2%)	0.021	828 (0%)	669 (0%)	0.007
Venous thromboembolism	6,564 (6%)	6,603 (6%)	0.000	7,760 (3%)	7,984 (3%)	0.007	980 (3%)	965 (3%)	0.003	2,860 (0%)	1,602 (0%)	0.034

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S5: Characteristics of unweighted populations in CPRD AURUM, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	348,052	332,790		1,976,163	594,262		1,510,323	54,102		2,014,161	1,335,671	
Age, median [Q25-Q75]	78 [76-82]	82 [79-86]	0.375	42 [32-55]	67 [56-73]	0.899	41 [31-53]	58 [50-82]	0.777	35 [27-45]	31 [25-37]	0.377
Sex: Female, N(%)	193,520 (56%)	186,481 (56%)	0.009	1,244,798 (63%)	324,259 (55%)	0.172	880,418 (58%)	32,310 (60%)	0.029	856,596 (43%)	625,195 (47%)	0.086
Years of prior history*, median [Q25-Q75]	23 [10-35]	26 [13-38]	0.081	15 [7-25]	21 [9-32]	0.261	13 [6-22]	19 [8-30]	0.308	8 [4-16]	7 [3-19]	0.012
Number of GP visits, median [Q25-Q75]	10 [5-17]	11 [6-18]	0.094	6 [2-11]	11 [6-17]	0.381	4 [1-10]	10 [5-17]	0.436	2 [0-5]	3 [1-7]	0.103
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.013	0[0-0]	0[0-0]	0.056	0[0-0]	0[0-0]	0.165	0[0-0]	0[0-0]	0.082
Comorbidities, N(%)**												
Anxiety	51,245 (15%)	49,410 (15%)	0.003	435,705 (22%)	122,087 (21%)	0.037	289,623 (19%)	10,441 (19%)	0.003	264,577 (13%)	218,144 (16%)	0.090
Asthma	36,625 (11%)	37,889 (11%)	0.028	501,289 (25%)	116,596 (20%)	0.138	294,101 (19%)	8,668 (16%)	0.090	137,548 (7%)	114,378 (9%)	0.065
Chronic kidney disease	71,413 (21%)	86,899 (26%)	0.133	55,136 (3%)	56,923 (10%)	0.285	24,372 (2%)	6,692 (12%)	0.431	18,579 (1%)	2,664 (0%)	0.097
COPD	28,829 (8%)	28,317 (9%)	0.008	29,793 (2%)	39,595 (7%)	0.263	13,430 (1%)	2,063 (4%)	0.194	10,501 (1%)	942 (0%)	0.083
Dementia	16,828 (5%)	16,138 (5%)	0.001	6,731 (0%)	3,720 (1%)	0.041	3,424 (0%)	1,323 (2%)	0.194	2,673 (0%)	119 (0%)	0.047
Depressive disorder	42,297 (12%)	37,831 (11%)	0.024	367,534 (19%)	112,753 (19%)	0.010	236,577 (16%)	9,046 (17%)	0.029	209,905 (10%)	155,037 (12%)	0.038
Diabetes	63,483 (18%)	61,929 (19%)	0.010	107,471 (5%)	98,676 (17%)	0.362	60,945 (4%)	5,817 (11%)	0.259	49,143 (2%)	12,040 (1%)	0.120
GERD	18,404 (5%)	20,204 (6%)	0.034	68,110 (3%)	32,288 (5%)	0.097	39,607 (3%)	2,506 (5%)	0.108	31,062 (2%)	21,599 (2%)	0.006
Heart failure	17,980 (5%)	20,430 (6%)	0.042	12,311 (1%)	13,120 (2%)	0.134	5,627 (0%)	1,529 (3%)	0.197	4,614 (0%)	448 (0%)	0.054
Hypertension	173,422 (50%)	186,367 (56%)	0.124	206,136 (10%)	200,762 (34%)	0.586	110,969 (7%)	15,536 (29%)	0.579	86,231 (4%)	19,649 (1%)	0.169
Hypothyroidism	31,595 (9%)	34,512 (10%)	0.044	78,935 (4%)	44,456 (7%)	0.150	43,876 (3%)	3,642 (7%)	0.179	30,913 (2%)	18,516 (1%)	0.012
Malignant neoplastic disease	69,247 (20%)	82,610 (25%)	0.118	60,370 (3%)	76,103 (13%)	0.367	29,693 (2%)	6,311 (12%)	0.392	22,630 (1%)	5,104 (0%)	0.086

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	16,111 (5%)	18,289 (5%)	0.040	20,304 (1%)	23,019 (4%)	0.185	7,409 (0%)	1,464 (3%)	0.177	5,738 (0%)	715 (0%)	0.056
Osteoporosis	30,053 (9%)	36,535 (11%)	0.079	18,282 (1%)	21,789 (4%)	0.184	8,852 (1%)	2,632 (5%)	0.265	6,904 (0%)	1,028 (0%)	0.058
Pneumonia	17,134 (5%)	16,297 (5%)	0.001	31,724 (2%)	17,567 (3%)	0.091	17,791 (1%)	1,658 (3%)	0.131	13,263 (1%)	7,793 (1%)	0.010
Rheumatoid arthritis	6,613 (2%)	6,693 (2%)	0.008	14,823 (1%)	12,878 (2%)	0.118	5,533 (0%)	667 (1%)	0.097	3,692 (0%)	891 (0%)	0.033
Stroke	15,325 (4%)	15,601 (5%)	0.014	16,962 (1%)	15,056 (3%)	0.130	7,431 (0%)	1,281 (2%)	0.158	6,019 (0%)	1,022 (0%)	0.051
Venous thromboembolism	19,679 (6%)	21,549 (6%)	0.034	28,882 (1%)	23,632 (4%)	0.155	13,639 (1%)	1,980 (4%)	0.185	12,941 (1%)	2,541 (0%)	0.070

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S6: Characteristics of weighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	40,084	40,014		111,674	111,393		124,883	124,955		196,235	195,302	
Age, median [Q25-Q75]	82 [78-86]	82 [78-86]	0.004	62 [50-69]	62 [50-69]	0.005	50 [41-58]	52 [41-58]	0.000	34 [26-42]	34 [26-43]	0.004
Sex: Female, N(%)	23,206 (58%)	23,175 (58%)	0.000	63,290 (57%)	63,079 (57%)	0.001	63,572 (51%)	63,955 (51%)	0.006	84,762 (43%)	85,083 (44%)	0.007
Years of prior history*, median [Q25-Q75]	17 [13-20]	18 [13-20]	0.001	17 [11-19]	17 [11-19]	0.005	17 [9-19]	17 [9-19]	0.000	13 [6-18]	14 [5-18]	0.006
Number of GP visits, median [Q25-Q75]	12 [7-20]	12 [8-20]	0.000	8 [3-15]	10 [5-15]	0.000	4 [0-10]	4 [1-10]	0.009	0 [0-5]	2 [0-5]	0.004
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.002	0[0-0]	0[0-0]	0.009	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.000
Comorbidities, N(%)**												
Anxiety	4,933 (12%)	4,920 (12%)	0.000	22,884 (20%)	22,299 (20%)	0.012	24,993 (20%)	24,695 (20%)	0.006	31,360 (16%)	30,689 (16%)	0.007
Asthma	3,911 (10%)	3,931 (10%)	0.002	18,514 (17%)	18,209 (16%)	0.006	17,431 (14%)	17,388 (14%)	0.001	14,817 (8%)	14,596 (7%)	0.003
Chronic kidney disease	8,998 (22%)	8,953 (22%)	0.002	6,848 (6%)	7,431 (7%)	0.022	2,120 (2%)	2,287 (2%)	0.010	922 (0%)	807 (0%)	0.009
COPD	3,439 (9%)	3,341 (8%)	0.008	5,972 (5%)	6,496 (6%)	0.021	2,089 (2%)	1,938 (2%)	0.010	734 (0%)	688 (0%)	0.004
Dementia	2,634 (7%)	2,332 (6%)	0.031	1,383 (1%)	865 (1%)	0.046	283 (0%)	313 (0%)	0.005	78 (0%)	134 (0%)	0.013
Depressive disorder	3,783 (9%)	3,705 (9%)	0.006	20,874 (19%)	20,246 (18%)	0.013	22,015 (18%)	21,682 (17%)	0.007	23,010 (12%)	22,630 (12%)	0.004
Diabetes	5,928 (15%)	5,802 (14%)	0.008	11,000 (10%)	11,657 (10%)	0.020	5,345 (4%)	5,651 (5%)	0.012	2,586 (1%)	2,366 (1%)	0.009
GERD	1,930 (5%)	1,970 (5%)	0.005	4,521 (4%)	4,479 (4%)	0.001	3,343 (3%)	3,346 (3%)	0.000	2,657 (1%)	2,614 (1%)	0.001
Heart failure	2,417 (6%)	2,205 (6%)	0.022	2,002 (2%)	2,029 (2%)	0.002	639 (1%)	614 (0%)	0.003	250 (0%)	207 (0%)	0.006
Hypertension	14,288 (36%)	14,282 (36%)	0.001	22,488 (20%)	22,695 (20%)	0.006	11,108 (9%)	11,313 (9%)	0.006	4,318 (2%)	4,619 (2%)	0.011
Hypothyroidism	3,151 (8%)	3,155 (8%)	0.001	6,321 (6%)	6,361 (6%)	0.002	4,134 (3%)	4,055 (3%)	0.004	2,475 (1%)	2,621 (1%)	0.007
Malignant neoplastic disease	8,302 (21%)	8,283 (21%)	0.000	8,661 (8%)	10,177 (9%)	0.050	3,813 (3%)	3,777 (3%)	0.002	1,614 (1%)	1,676 (1%)	0.004

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	2,119 (5%)	1,984 (5%)	0.015	3,308 (3%)	3,740 (3%)	0.023	1,193 (1%)	1,267 (1%)	0.006	430 (0%)	343 (0%)	0.010
Osteoporosis	3,702 (9%)	3,670 (9%)	0.002	3,154 (3%)	3,179 (3%)	0.002	1,038 (1%)	1,030 (1%)	0.001	363 (0%)	375 (0%)	0.002
Pneumonia	1,654 (4%)	1,452 (4%)	0.026	2,424 (2%)	2,211 (2%)	0.013	1,401 (1%)	1,314 (1%)	0.007	1,035 (1%)	1,055 (1%)	0.002
Rheumatoid arthritis	637 (2%)	624 (2%)	0.002	1,618 (1%)	1,818 (2%)	0.015	551 (0%)	760 (1%)	0.023	280 (0%)	157 (0%)	0.019
Stroke	2,103 (5%)	1,866 (5%)	0.027	2,468 (2%)	2,512 (2%)	0.003	916 (1%)	981 (1%)	0.006	385 (0%)	356 (0%)	0.003
Venous thromboembolism	1,732 (4%)	1,648 (4%)	0.010	2,639 (2%)	2,596 (2%)	0.002	1,466 (1%)	1,638 (1%)	0.012	959 (0%)	518 (0%)	0.036

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S7: Characteristics of unweighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	169,100	118,507		583,399	486,619		417,996	462,832		469,876	550,437	
Age, median [Q25-Q75]	78 [76-82]	84 [81-87]	0.550	45 [33-58]	71 [66-75]	1.117	42 [31-55]	56 [52-61]	0.546	37 [28-49]	35 [27-43]	0.250
Sex: Female, N(%)	93,719 (55%)	68,258 (58%)	0.044	344,747 (59%)	262,113 (54%)	0.106	230,567 (55%)	230,957 (50%)	0.105	208,184 (44%)	249,603 (45%)	0.021
Years of prior history*, median [Q25-Q75]	17 [13-20]	18 [14-20]	0.023	17 [10-19]	17 [13-20]	0.085	17 [9-19]	17 [10-19]	0.055	13 [6-18]	14 [6-18]	0.020
Number of GP visits, median [Q25-Q75]	11 [6-18]	14 [9-21]	0.180	5 [1-11]	11 [6-17]	0.414	3 [0-8]	6 [2-12]	0.239	0 [0-4]	2 [0-6]	0.115
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.052	0[0-0]	0[0-0]	0.069	0[0-0]	0[0-0]	0.016	0[0-0]	0[0-0]	0.044
Comorbidities, N(%)**												
Anxiety	20,097 (12%)	14,878 (13%)	0.020	132,282 (23%)	78,442 (16%)	0.166	83,022 (20%)	83,629 (18%)	0.046	65,306 (14%)	88,512 (16%)	0.061
Asthma	15,573 (9%)	12,090 (10%)	0.034	119,520 (20%)	61,821 (13%)	0.210	68,746 (16%)	52,312 (11%)	0.149	30,811 (7%)	46,774 (8%)	0.074
Chronic kidney disease	29,343 (17%)	29,934 (25%)	0.194	15,484 (3%)	49,550 (10%)	0.311	6,676 (2%)	8,145 (2%)	0.013	4,746 (1%)	1,376 (0%)	0.096
COPD	14,248 (8%)	9,602 (8%)	0.012	12,247 (2%)	39,371 (8%)	0.275	5,114 (1%)	6,699 (1%)	0.020	3,716 (1%)	953 (0%)	0.089
Dementia	6,678 (4%)	6,860 (6%)	0.086	1,986 (0%)	6,557 (1%)	0.110	1,188 (0%)	636 (0%)	0.032	774 (0%)	159 (0%)	0.044
Depressive disorder	16,339 (10%)	10,720 (9%)	0.021	111,568 (19%)	72,063 (15%)	0.115	66,705 (16%)	77,560 (17%)	0.022	50,341 (11%)	64,215 (12%)	0.030
Diabetes	23,579 (14%)	17,895 (15%)	0.033	29,125 (5%)	66,688 (14%)	0.303	12,407 (3%)	25,465 (6%)	0.126	9,225 (2%)	5,012 (1%)	0.089
GERD	7,610 (5%)	6,174 (5%)	0.033	18,053 (3%)	22,884 (5%)	0.083	8,864 (2%)	15,586 (3%)	0.076	5,920 (1%)	8,482 (2%)	0.024
Heart failure	7,475 (4%)	7,118 (6%)	0.071	4,013 (1%)	12,939 (3%)	0.154	1,732 (0%)	2,410 (1%)	0.016	1,274 (0%)	293 (0%)	0.054
Hypertension	56,298 (33%)	44,709 (38%)	0.093	58,662 (10%)	139,644 (29%)	0.485	24,457 (6%)	64,451 (14%)	0.273	16,440 (3%)	11,991 (2%)	0.080
Hypothyroidism	12,126 (7%)	9,825 (8%)	0.042	22,026 (4%)	31,923 (7%)	0.126	10,616 (3%)	19,001 (4%)	0.087	6,676 (1%)	7,790 (1%)	0.000
Malignant neoplastic disease	30,687 (18%)	26,725 (23%)	0.110	19,781 (3%)	65,779 (14%)	0.370	8,870 (2%)	19,474 (4%)	0.119	5,916 (1%)	4,164 (1%)	0.050

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	7,707 (5%)	6,298 (5%)	0.035	8,409 (1%)	20,200 (4%)	0.165	2,665 (1%)	5,556 (1%)	0.059	2,003 (0%)	501 (0%)	0.066
Osteoporosis	12,736 (8%)	11,677 (10%)	0.082	5,836 (1%)	23,843 (5%)	0.232	2,580 (1%)	4,797 (1%)	0.046	1,855 (0%)	637 (0%)	0.055
Pneumonia	5,401 (3%)	4,381 (4%)	0.028	7,250 (1%)	12,120 (2%)	0.092	3,822 (1%)	4,889 (1%)	0.014	2,522 (1%)	2,797 (1%)	0.004
Rheumatoid arthritis	2,565 (2%)	1,833 (2%)	0.002	4,013 (1%)	9,000 (2%)	0.104	1,294 (0%)	2,566 (1%)	0.037	877 (0%)	316 (0%)	0.037
Stroke	7,214 (4%)	5,843 (5%)	0.032	5,886 (1%)	14,921 (3%)	0.146	2,170 (1%)	3,998 (1%)	0.042	1,591 (0%)	620 (0%)	0.048
Venous thromboembolism	6,188 (4%)	5,096 (4%)	0.033	7,342 (1%)	14,077 (3%)	0.115	3,067 (1%)	6,732 (1%)	0.069	2,611 (1%)	1,018 (0%)	0.061

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S8: Characteristics of weighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	29,163	29,082		82,091	82,226		116,090	115,912		70,312	70,823	
Age, median [Q25-Q75]	82 [79-87]	82 [80-87]	0.002	64 [53-71]	64 [53-70]	0.001	52 [42-58]	52 [42-58]	0.003	42 [39-48]	42 [38-48]	0.000
Sex: Female, N(%)	17,126 (59%)	17,076 (59%)	0.000	45,269 (55%)	45,487 (55%)	0.003	58,599 (50%)	58,799 (51%)	0.005	29,480 (42%)	29,458 (42%)	0.007
Years of prior history*, median [Q25-Q75]	17 [13-20]	17 [14-20]	0.001	17 [11-19]	17 [11-19]	0.002	17 [9-19]	17 [8-19]	0.001	14 [7-18]	14 [6-18]	0.001
Number of GP visits, median [Q25-Q75]	12 [7-21]	12 [8-20]	0.005	8 [3-16]	10 [5-16]	0.002	4 [0-10]	4 [1-10]	0.014	0 [0-6]	2 [0-6]	0.003
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.005	0[0-0]	0[0-0]	0.005	0[0-0]	0[0-0]	0.004	0[0-0]	0[0-0]	0.000
Comorbidities, N(%)**												
Anxiety	3,481 (12%)	3,496 (12%)	0.003	16,202 (20%)	15,970 (19%)	0.008	23,083 (20%)	22,695 (20%)	0.008	12,154 (17%)	11,947 (17%)	0.011
Asthma	2,863 (10%)	2,870 (10%)	0.002	13,358 (16%)	13,192 (16%)	0.006	16,057 (14%)	16,029 (14%)	0.000	4,767 (7%)	4,744 (7%)	0.003
Chronic kidney disease	6,959 (24%)	6,928 (24%)	0.001	5,850 (7%)	6,384 (8%)	0.024	2,031 (2%)	2,104 (2%)	0.005	616 (1%)	517 (1%)	0.016
COPD	2,556 (9%)	2,485 (9%)	0.008	5,069 (6%)	5,523 (7%)	0.022	1,969 (2%)	1,874 (2%)	0.006	539 (1%)	478 (1%)	0.011
Dementia	2,042 (7%)	1,794 (6%)	0.034	1,319 (2%)	826 (1%)	0.053	263 (0%)	265 (0%)	0.000	59 (0%)	111 (0%)	0.021
Depressive disorder	2,641 (9%)	2,608 (9%)	0.003	14,875 (18%)	14,558 (18%)	0.011	20,497 (18%)	20,298 (18%)	0.004	10,492 (15%)	10,218 (14%)	0.014
Diabetes	4,346 (15%)	4,273 (15%)	0.006	8,933 (11%)	9,524 (12%)	0.022	5,139 (4%)	5,350 (5%)	0.009	1,459 (2%)	1,421 (2%)	0.005
GERD	1,366 (5%)	1,380 (5%)	0.003	3,514 (4%)	3,414 (4%)	0.006	3,153 (3%)	3,136 (3%)	0.001	1,258 (2%)	1,297 (2%)	0.003
Heart failure	1,844 (6%)	1,699 (6%)	0.020	1,793 (2%)	1,720 (2%)	0.006	613 (1%)	601 (1%)	0.001	177 (0%)	151 (0%)	0.008
Hypertension	10,466 (36%)	10,581 (36%)	0.010	17,843 (22%)	18,115 (22%)	0.007	10,489 (9%)	10,714 (9%)	0.007	3,119 (4%)	3,233 (5%)	0.006
Hypothyroidism	2,338 (8%)	2,360 (8%)	0.004	4,857 (6%)	4,837 (6%)	0.001	3,897 (3%)	3,742 (3%)	0.007	1,432 (2%)	1,412 (2%)	0.003
Malignant neoplastic disease	6,210 (21%)	6,164 (21%)	0.002	7,090 (9%)	8,270 (10%)	0.049	3,588 (3%)	3,590 (3%)	0.000	1,059 (2%)	1,117 (2%)	0.006
Myocardial infarction	1,569 (5%)	1,500 (5%)	0.010	2,720 (3%)	3,083 (4%)	0.024	1,155 (1%)	1,202 (1%)	0.004	319 (0%)	242 (0%)	0.018

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Osteoporosis	2,838 (10%)	2,846 (10%)	0.002	2,715 (3%)	2,668 (3%)	0.004	944 (1%)	986 (1%)	0.004	252 (0%)	281 (0%)	0.006
Pneumonia	1,287 (4%)	1,147 (4%)	0.024	1,997 (2%)	1,883 (2%)	0.009	1,307 (1%)	1,245 (1%)	0.005	487 (1%)	473 (1%)	0.003
Rheumatoid arthritis	464 (2%)	445 (2%)	0.005	1,280 (2%)	1,491 (2%)	0.020	514 (0%)	743 (1%)	0.027	184 (0%)	120 (0%)	0.020
Stroke	1,600 (5%)	1,421 (5%)	0.027	2,073 (3%)	2,122 (3%)	0.003	873 (1%)	963 (1%)	0.009	263 (0%)	257 (0%)	0.002
Venous thromboembolism	1,339 (5%)	1,191 (4%)	0.024	2,112 (3%)	2,219 (3%)	0.008	1,407 (1%)	1,572 (1%)	0.013	590 (1%)	297 (0%)	0.053

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S9: Characteristics of unweighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	168,972	82,406		582,791	302,999		418,184	423,876		485,154	147,744	
Age, median [Q25-Q75]	78 [76-82]	84 [82-88]	0.657	45 [33-58]	71 [66-76]	1.153	42 [31-55]	56 [52-61]	0.560	37 [28-49]	44 [41-48]	0.262
Sex: Female, N(%)	93,648 (55%)	47,915 (58%)	0.055	344,408 (59%)	162,753 (54%)	0.109	230,680 (55%)	210,283 (50%)	0.111	215,080 (44%)	64,999 (44%)	0.007
Years of prior history*, median [Q25-Q75]	17 [13-20]	17 [14-20]	0.016	17 [10-19]	17 [13-19]	0.090	17 [9-19]	17 [10-19]	0.047	13 [6-18]	14 [7-18]	0.054
Number of GP visits, median [Q25-Q75]	11 [6-18]	14 [9-21]	0.177	5 [1-11]	11 [7-18]	0.451	3 [0-8]	6 [2-12]	0.245	0 [0-4]	3 [0-7]	0.174
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.053	0[0-0]	0[0-0]	0.074	0[0-0]	0[0-0]	0.018	0[0-0]	0[0-0]	0.029
Comorbidities, N(%)**												
Anxiety	20,087 (12%)	9,923 (12%)	0.005	132,145 (23%)	49,010 (16%)	0.165	83,054 (20%)	75,258 (18%)	0.054	66,918 (14%)	24,601 (17%)	0.080
Asthma	15,567 (9%)	8,491 (10%)	0.037	119,442 (20%)	39,988 (13%)	0.196	68,792 (16%)	48,034 (11%)	0.148	31,538 (7%)	10,517 (7%)	0.025
Chronic kidney disease	29,280 (17%)	21,564 (26%)	0.216	15,435 (3%)	34,168 (11%)	0.344	6,675 (2%)	7,553 (2%)	0.014	4,947 (1%)	739 (1%)	0.060
COPD	14,234 (8%)	6,837 (8%)	0.005	12,242 (2%)	27,472 (9%)	0.307	5,122 (1%)	6,334 (1%)	0.023	3,834 (1%)	640 (0%)	0.046
Dementia	6,664 (4%)	4,773 (6%)	0.086	1,998 (0%)	5,124 (2%)	0.135	1,201 (0%)	548 (0%)	0.035	831 (0%)	126 (0%)	0.024
Depressive disorder	16,333 (10%)	6,998 (8%)	0.041	111,526 (19%)	45,154 (15%)	0.113	66,763 (16%)	70,703 (17%)	0.019	51,687 (11%)	21,711 (15%)	0.122
Diabetes	23,547 (14%)	12,387 (15%)	0.031	29,089 (5%)	43,647 (14%)	0.322	12,392 (3%)	24,219 (6%)	0.135	9,455 (2%)	2,254 (2%)	0.032
GERD	7,613 (5%)	4,054 (5%)	0.020	18,044 (3%)	14,294 (5%)	0.084	8,869 (2%)	14,447 (3%)	0.079	6,051 (1%)	2,883 (2%)	0.056
Heart failure	7,468 (4%)	5,058 (6%)	0.077	4,016 (1%)	9,180 (3%)	0.174	1,738 (0%)	2,308 (1%)	0.019	1,316 (0%)	191 (0%)	0.032
Hypertension	56,262 (33%)	31,217 (38%)	0.096	58,578 (10%)	88,833 (29%)	0.499	24,450 (6%)	59,554 (14%)	0.277	16,971 (3%)	6,761 (5%)	0.055
Hypothyroidism	12,132 (7%)	7,042 (9%)	0.051	21,999 (4%)	20,521 (7%)	0.134	10,622 (3%)	17,382 (4%)	0.087	6,848 (1%)	3,245 (2%)	0.059
Malignant neoplastic disease	30,670 (18%)	18,950 (23%)	0.120	19,707 (3%)	42,687 (14%)	0.386	8,893 (2%)	17,903 (4%)	0.120	6,143 (1%)	2,192 (1%)	0.019

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	7,704 (5%)	4,500 (5%)	0.041	8,413 (1%)	13,456 (4%)	0.178	2,660 (1%)	5,280 (1%)	0.063	2,057 (0%)	330 (0%)	0.035
Osteoporosis	12,732 (8%)	8,564 (10%)	0.100	5,847 (1%)	16,117 (5%)	0.249	2,576 (1%)	4,330 (1%)	0.045	1,923 (0%)	408 (0%)	0.021
Pneumonia	5,401 (3%)	3,231 (4%)	0.039	7,241 (1%)	8,604 (3%)	0.113	3,814 (1%)	4,527 (1%)	0.016	2,615 (1%)	921 (1%)	0.011
Rheumatoid arthritis	2,559 (2%)	1,257 (2%)	0.001	4,017 (1%)	5,999 (2%)	0.113	1,286 (0%)	2,405 (1%)	0.039	918 (0%)	191 (0%)	0.015
Stroke	7,200 (4%)	4,193 (5%)	0.039	5,885 (1%)	10,213 (3%)	0.162	2,167 (1%)	3,789 (1%)	0.045	1,625 (0%)	369 (0%)	0.016
Venous thromboembolism	6,185 (4%)	3,550 (4%)	0.033	7,335 (1%)	9,466 (3%)	0.128	3,072 (1%)	6,339 (1%)	0.073	2,712 (1%)	460 (0%)	0.038

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S10: Characteristics of weighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	15,295	15,304		60,813	61,113		24,008	24,157		151,713	152,100	
Age, median [Q25-Q75]	80 [77-85]	80 [77-85]	0.000	64 [53-70]	66 [52-70]	0.008	52 [44-58]	52 [44-58]	0.004	32 [24-38]	32 [24-38]	0.003
Sex: Female, N(%)	8,644 (57%)	8,657 (57%)	0.001	34,612 (57%)	34,776 (57%)	0.000	12,846 (54%)	12,993 (54%)	0.006	66,947 (44%)	67,555 (44%)	0.006
Years of prior history*, median [Q25-Q75]	17 [12-20]	18 [12-21]	0.001	17 [12-19]	17 [11-19]	0.003	17 [11-19]	17 [11-19]	0.004	13 [6-18]	14 [5-18]	0.001
Number of GP visits, median [Q25-Q75]	12 [7-20]	12 [8-20]	0.001	8 [3-15]	8 [4-15]	0.009	4 [0-10]	4 [1-10]	0.006	0 [0-5]	2 [0-5]	0.007
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.006	0[0-0]	0[0-0]	0.004	0[0-0]	0[0-0]	0.001	0[0-0]	0[0-0]	0.013
Comorbidities, N(%)**												
Anxiety	1,969 (13%)	1,940 (13%)	0.006	11,790 (19%)	11,914 (19%)	0.003	5,342 (22%)	5,235 (22%)	0.014	24,455 (16%)	23,928 (16%)	0.011
Asthma	1,498 (10%)	1,488 (10%)	0.002	9,126 (15%)	9,093 (15%)	0.004	2,926 (12%)	2,985 (12%)	0.005	12,195 (8%)	11,929 (8%)	0.007
Chronic kidney disease	3,112 (20%)	3,057 (20%)	0.009	3,563 (6%)	3,764 (6%)	0.013	398 (2%)	407 (2%)	0.002	468 (0%)	346 (0%)	0.016
COPD	1,190 (8%)	1,151 (8%)	0.010	3,222 (5%)	3,237 (5%)	0.000	293 (1%)	274 (1%)	0.008	315 (0%)	250 (0%)	0.010
Dementia	1,087 (7%)	864 (6%)	0.060	646 (1%)	355 (1%)	0.053	55 (0%)	80 (0%)	0.019	21 (0%)	28 (0%)	0.004
Depressive disorder	1,527 (10%)	1,496 (10%)	0.007	10,709 (18%)	10,912 (18%)	0.006	4,382 (18%)	4,343 (18%)	0.007	17,112 (11%)	16,594 (11%)	0.012
Diabetes	2,193 (14%)	2,173 (14%)	0.004	6,057 (10%)	6,199 (10%)	0.006	883 (4%)	858 (4%)	0.007	1,609 (1%)	1,271 (1%)	0.023
GERD	880 (6%)	872 (6%)	0.003	2,440 (4%)	2,505 (4%)	0.004	659 (3%)	706 (3%)	0.011	1,935 (1%)	1,961 (1%)	0.001
Heart failure	857 (6%)	748 (5%)	0.032	1,097 (2%)	933 (2%)	0.022	119 (0%)	77 (0%)	0.027	107 (0%)	77 (0%)	0.008
Hypertension	5,338 (35%)	5,312 (35%)	0.004	12,828 (21%)	12,901 (21%)	0.000	2,597 (11%)	2,645 (11%)	0.004	2,260 (1%)	2,369 (2%)	0.006
Hypothyroidism	1,129 (7%)	1,067 (7%)	0.016	3,476 (6%)	3,416 (6%)	0.005	935 (4%)	954 (4%)	0.003	1,772 (1%)	1,653 (1%)	0.008
Malignant neoplastic disease	3,126 (20%)	3,097 (20%)	0.005	5,084 (8%)	5,529 (9%)	0.024	864 (4%)	922 (4%)	0.011	877 (1%)	902 (1%)	0.002
Myocardial infarction	764 (5%)	681 (4%)	0.026	1,797 (3%)	1,853 (3%)	0.005	230 (1%)	186 (1%)	0.020	189 (0%)	128 (0%)	0.013

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Osteoporosis	1,312 (9%)	1,225 (8%)	0.021	1,775 (3%)	1,805 (3%)	0.002	251 (1%)	282 (1%)	0.012	154 (0%)	135 (0%)	0.004
Pneumonia	573 (4%)	475 (3%)	0.035	1,232 (2%)	1,107 (2%)	0.016	256 (1%)	244 (1%)	0.005	744 (0%)	698 (0%)	0.005
Rheumatoid arthritis	248 (2%)	234 (2%)	0.008	854 (1%)	945 (2%)	0.012	113 (0%)	102 (0%)	0.007	165 (0%)	62 (0%)	0.025
Stroke	739 (5%)	650 (4%)	0.028	1,291 (2%)	1,297 (2%)	0.000	172 (1%)	129 (1%)	0.023	204 (0%)	138 (0%)	0.013
Venous thromboembolism	640 (4%)	622 (4%)	0.006	1,355 (2%)	1,347 (2%)	0.002	273 (1%)	256 (1%)	0.008	604 (0%)	288 (0%)	0.039

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S11: Characteristics of unweighted populations in CPRD GOLD, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	169,459	32,755		584,309	180,670		416,549	36,748		465,326	365,096	
Age, median [Q25-Q75]	78 [76-82]	81 [79-86]	0.306	45 [33-58]	70 [66-74]	1.059	42 [31-55]	54 [50-60]	0.407	37 [28-49]	31 [24-37]	0.445
Sex: Female, N(%)	93,939 (55%)	18,415 (56%)	0.016	345,249 (59%)	97,747 (54%)	0.101	229,807 (55%)	19,649 (53%)	0.034	206,080 (44%)	167,510 (46%)	0.032
Years of prior history*, median [Q25-Q75]	17 [13-20]	18 [13-21]	0.017	17 [10-19]	17 [12-19]	0.076	17 [9-19]	18 [12-20]	0.153	13 [6-18]	15 [6-18]	0.018
Number of GP visits, median [Q25-Q75]	11 [6-18]	14 [9-21]	0.172	5 [1-11]	10 [6-16]	0.348	3 [0-8]	6 [2-11]	0.183	0 [0-4]	2 [0-6]	0.092
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.046	0[0-0]	0[0-0]	0.061	0[0-0]	0[0-0]	0.005	0[0-0]	0[0-0]	0.045
Comorbidities, N(%)**												
Anxiety	20,145 (12%)	4,336 (13%)	0.041	132,397 (23%)	28,697 (16%)	0.172	82,752 (20%)	8,004 (22%)	0.047	64,849 (14%)	59,094 (16%)	0.063
Asthma	15,611 (9%)	3,250 (10%)	0.024	119,679 (20%)	21,396 (12%)	0.236	68,556 (16%)	4,053 (11%)	0.158	30,579 (7%)	33,240 (9%)	0.094
Chronic kidney disease	29,378 (17%)	7,430 (23%)	0.134	15,504 (3%)	15,033 (8%)	0.251	6,636 (2%)	566 (2%)	0.004	4,692 (1%)	578 (0%)	0.112
COPD	14,265 (8%)	2,417 (7%)	0.039	12,276 (2%)	11,545 (6%)	0.214	5,085 (1%)	348 (1%)	0.026	3,657 (1%)	285 (0%)	0.108
Dementia	6,705 (4%)	1,895 (6%)	0.085	2,008 (0%)	1,375 (1%)	0.056	1,168 (0%)	87 (0%)	0.009	746 (0%)	29 (0%)	0.053
Depressive disorder	16,385 (10%)	3,310 (10%)	0.015	111,708 (19%)	26,294 (15%)	0.122	66,510 (16%)	6,569 (18%)	0.051	49,969 (11%)	39,181 (11%)	0.000
Diabetes	23,614 (14%)	4,882 (15%)	0.028	29,200 (5%)	22,552 (12%)	0.267	12,338 (3%)	1,191 (3%)	0.016	9,143 (2%)	2,507 (1%)	0.112
GERD	7,655 (5%)	2,012 (6%)	0.072	18,069 (3%)	8,525 (5%)	0.084	8,839 (2%)	1,093 (3%)	0.054	5,864 (1%)	5,213 (1%)	0.015
Heart failure	7,502 (4%)	1,782 (5%)	0.047	4,026 (1%)	3,669 (2%)	0.116	1,715 (0%)	100 (0%)	0.024	1,236 (0%)	98 (0%)	0.063
Hypertension	56,411 (33%)	11,995 (37%)	0.070	58,749 (10%)	49,763 (28%)	0.459	24,301 (6%)	4,679 (13%)	0.239	16,363 (4%)	4,776 (1%)	0.144
Hypothyroidism	12,163 (7%)	2,373 (7%)	0.003	22,061 (4%)	11,106 (6%)	0.109	10,565 (3%)	1,553 (4%)	0.094	6,617 (1%)	4,180 (1%)	0.025
Malignant neoplastic disease	30,728 (18%)	6,972 (21%)	0.079	19,832 (3%)	22,617 (13%)	0.342	8,827 (2%)	1,522 (4%)	0.116	5,804 (1%)	1,796 (0%)	0.081

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	7,716 (5%)	1,603 (5%)	0.016	8,437 (1%)	6,621 (4%)	0.141	2,644 (1%)	271 (1%)	0.012	1,985 (0%)	154 (0%)	0.080
Osteoporosis	12,776 (8%)	2,770 (8%)	0.034	5,867 (1%)	7,531 (4%)	0.200	2,558 (1%)	458 (1%)	0.066	1,841 (0%)	205 (0%)	0.072
Pneumonia	5,415 (3%)	1,032 (3%)	0.003	7,266 (1%)	3,418 (2%)	0.052	3,790 (1%)	345 (1%)	0.003	2,512 (1%)	1,696 (0%)	0.011
Rheumatoid arthritis	2,566 (2%)	512 (2%)	0.004	4,018 (1%)	2,929 (2%)	0.087	1,276 (0%)	154 (0%)	0.019	881 (0%)	111 (0%)	0.048
Stroke	7,203 (4%)	1,490 (5%)	0.015	5,907 (1%)	4,632 (3%)	0.117	2,152 (1%)	199 (1%)	0.003	1,578 (0%)	225 (0%)	0.062
Venous thromboembolism	6,198 (4%)	1,389 (4%)	0.030	7,333 (1%)	4,490 (2%)	0.091	3,056 (1%)	370 (1%)	0.029	2,585 (1%)	496 (0%)	0.072

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S12: Characteristics of weighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	58,342	58,535		235,543	235,780		344,520	342,678		413,428	412,893	
Age, median [Q25-Q75]	86 [83-89]	86 [82-89]	0.002	68 [63-73]	68 [63-74]	0.003	52 [46-58]	52 [46-59]	0.000	36 [28-44]	36 [28-44]	0.002
Sex: Female, N(%)	36,115 (62%)	36,214 (62%)	0.001	128,806 (55%)	129,053 (55%)	0.001	164,316 (48%)	163,188 (48%)	0.001	188,426 (46%)	188,471 (46%)	0.001
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.001	15 [15-15]	15 [15-15]	0.000	15 [15-15]	15 [15-15]	0.003	15 [13-16]	15 [13-16]	0.004
Number of GP visits, median [Q25-Q75]	8 [3-15]	8 [4-15]	0.007	4 [0-9]	4 [1-9]	0.014	0 [0-6]	2 [0-6]	0.001	0 [0-4]	0 [0-5]	0.014
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.006	0[0-0]	0[0-0]	0.002	0[0-0]	0[0-0]	0.004	0[0-0]	0[0-0]	0.009
Comorbidities, N(%)**												
Anxiety	11,384 (20%)	11,467 (20%)	0.002	52,314 (22%)	52,262 (22%)	0.001	81,514 (24%)	80,775 (24%)	0.002	84,856 (21%)	83,145 (20%)	0.010
Asthma	3,708 (6%)	3,627 (6%)	0.007	10,973 (5%)	10,703 (5%)	0.006	14,915 (4%)	14,492 (4%)	0.005	19,969 (5%)	19,628 (5%)	0.004
Chronic kidney disease	18,755 (32%)	18,803 (32%)	0.001	21,147 (9%)	21,249 (9%)	0.001	7,411 (2%)	7,335 (2%)	0.001	3,293 (1%)	2,846 (1%)	0.012
COPD	7,008 (12%)	6,983 (12%)	0.002	17,160 (7%)	16,784 (7%)	0.006	8,852 (3%)	8,622 (3%)	0.003	3,210 (1%)	3,215 (1%)	0.000
Dementia	5,686 (10%)	5,812 (10%)	0.006	4,628 (2%)	4,425 (2%)	0.006	1,247 (0%)	1,229 (0%)	0.001	673 (0%)	689 (0%)	0.001
Depressive disorder	10,960 (19%)	11,020 (19%)	0.001	34,232 (15%)	34,571 (15%)	0.004	34,243 (10%)	32,427 (9%)	0.016	23,679 (6%)	23,024 (6%)	0.007
Diabetes	14,529 (25%)	14,269 (24%)	0.012	39,335 (17%)	39,133 (17%)	0.003	25,964 (8%)	26,135 (8%)	0.003	14,136 (3%)	14,091 (3%)	0.000
GERD	6,898 (12%)	6,871 (12%)	0.003	21,088 (9%)	21,212 (9%)	0.002	16,959 (5%)	17,024 (5%)	0.002	10,569 (3%)	10,903 (3%)	0.005
Heart failure	9,320 (16%)	9,141 (16%)	0.010	10,251 (4%)	10,196 (4%)	0.001	4,001 (1%)	3,743 (1%)	0.007	1,606 (0%)	1,565 (0%)	0.002
Hypertension	38,140 (65%)	37,917 (65%)	0.013	96,384 (41%)	96,118 (41%)	0.003	58,596 (17%)	57,241 (17%)	0.008	22,160 (5%)	22,739 (6%)	0.006
Hypothyroidism	7,350 (13%)	7,236 (12%)	0.007	24,054 (10%)	24,260 (10%)	0.003	21,956 (6%)	21,573 (6%)	0.003	17,452 (4%)	17,758 (4%)	0.004
Malignant neoplastic disease	15,402 (26%)	15,230 (26%)	0.009	37,052 (16%)	38,490 (16%)	0.016	19,765 (6%)	19,599 (6%)	0.001	9,413 (2%)	8,865 (2%)	0.009

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Myocardial infarction	2,258 (4%)	2,235 (4%)	0.003	5,972 (3%)	5,812 (2%)	0.005	3,615 (1%)	3,448 (1%)	0.004	1,362 (0%)	1,364 (0%)	0.000
Osteoporosis	9,270 (16%)	9,151 (16%)	0.007	18,870 (8%)	19,036 (8%)	0.002	7,126 (2%)	7,039 (2%)	0.001	2,254 (1%)	2,040 (0%)	0.007
Pneumonia	6,643 (11%)	6,563 (11%)	0.005	15,630 (7%)	15,557 (7%)	0.002	14,821 (4%)	14,580 (4%)	0.002	14,171 (3%)	14,083 (3%)	0.001
Rheumatoid arthritis	765 (1%)	727 (1%)	0.006	2,274 (1%)	2,286 (1%)	0.000	1,544 (0%)	1,592 (0%)	0.002	754 (0%)	646 (0%)	0.006
Stroke	5,119 (9%)	5,043 (9%)	0.006	8,750 (4%)	7,706 (3%)	0.024	4,343 (1%)	4,147 (1%)	0.005	1,828 (0%)	1,840 (0%)	0.001
Venous thromboembolism	2,469 (4%)	2,418 (4%)	0.005	4,745 (2%)	4,419 (2%)	0.010	3,365 (1%)	2,982 (1%)	0.011	1,931 (0%)	1,777 (0%)	0.005

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S13: Characteristics of unweighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	223,962	89,941		433,151	819,590		869,497	954,232		1,061,634	880,950	
Age, median [Q25-Q75]	85 [82-88]	87 [83-90]	0.253	67 [62-72]	72 [65-78]	0.391	48 [43-57]	54 [49-58]	0.192	38 [28-51]	37 [29-43]	0.246
Sex: Female, N(%)	136,888 (61%)	56,235 (63%)	0.029	237,494 (55%)	443,019 (54%)	0.016	409,207 (47%)	462,599 (48%)	0.028	488,499 (46%)	408,555 (46%)	0.007
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.051	15 [15-15]	15 [15-15]	0.185	15 [15-15]	15 [15-15]	0.246	15 [10-16]	15 [15-16]	0.195
Number of GP visits, median [Q25-Q75]	7 [2-13]	10 [5-18]	0.251	3 [0-8]	6 [2-11]	0.168	1 [0-5]	2 [0-7]	0.105	0 [0-4]	2 [0-6]	0.060
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-1]	0.070	0[0-0]	0[0-1]	0.042	0[0-0]	0[0-1]	0.073	0[0-0]	0[0-1]	0.123
Comorbidities, N(%)**												
Anxiety	41,908 (19%)	18,117 (20%)	0.036	97,857 (23%)	181,286 (22%)	0.011	197,922 (23%)	233,123 (24%)	0.039	203,019 (19%)	186,562 (21%)	0.051
Asthma	13,371 (6%)	5,841 (6%)	0.022	19,809 (5%)	41,966 (5%)	0.025	36,811 (4%)	42,221 (4%)	0.009	45,374 (4%)	46,776 (5%)	0.049
Chronic kidney disease	63,041 (28%)	31,132 (35%)	0.140	32,450 (7%)	101,792 (12%)	0.165	22,454 (3%)	20,303 (2%)	0.030	20,368 (2%)	5,314 (1%)	0.118
COPD	24,459 (11%)	11,292 (13%)	0.051	28,780 (7%)	67,795 (8%)	0.062	19,020 (2%)	27,451 (3%)	0.044	15,415 (1%)	5,836 (1%)	0.077
Dementia	16,743 (7%)	10,455 (12%)	0.142	7,404 (2%)	19,153 (2%)	0.045	6,009 (1%)	2,787 (0%)	0.057	5,866 (1%)	1,310 (0%)	0.068
Depressive disorder	38,148 (17%)	18,040 (20%)	0.078	61,308 (14%)	124,929 (15%)	0.031	77,039 (9%)	98,723 (10%)	0.050	66,864 (6%)	49,608 (6%)	0.028
Diabetes	51,964 (23%)	22,968 (26%)	0.054	65,572 (15%)	155,362 (19%)	0.102	60,254 (7%)	78,356 (8%)	0.048	49,331 (5%)	29,768 (3%)	0.065
GERD	25,376 (11%)	10,728 (12%)	0.019	34,715 (8%)	87,626 (11%)	0.092	37,828 (4%)	53,565 (6%)	0.058	31,600 (3%)	23,657 (3%)	0.018
Heart failure	28,538 (13%)	16,202 (18%)	0.147	16,697 (4%)	42,924 (5%)	0.066	11,909 (1%)	10,469 (1%)	0.025	10,738 (1%)	2,776 (0%)	0.086
Hypertension	138,631 (62%)	60,128 (67%)	0.104	157,290 (36%)	390,610 (48%)	0.231	125,239 (14%)	188,390 (20%)	0.142	97,835 (9%)	43,351 (5%)	0.168

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Hypothyroidism	26,875 (12%)	11,473 (13%)	0.023	42,245 (10%)	89,496 (11%)	0.038	50,396 (6%)	64,873 (7%)	0.041	49,279 (5%)	38,477 (4%)	0.013
Malignant neoplastic disease	54,714 (24%)	24,173 (27%)	0.056	81,394 (19%)	151,745 (19%)	0.007	48,971 (6%)	59,566 (6%)	0.026	37,755 (4%)	18,021 (2%)	0.092
Myocardial infarction	7,886 (4%)	3,642 (4%)	0.028	9,894 (2%)	22,773 (3%)	0.031	7,570 (1%)	11,483 (1%)	0.033	6,171 (1%)	2,459 (0%)	0.046
Osteoporosis	32,239 (14%)	14,846 (17%)	0.058	29,512 (7%)	81,707 (10%)	0.114	16,393 (2%)	21,679 (2%)	0.027	14,373 (1%)	3,343 (0%)	0.105
Pneumonia	22,254 (10%)	10,959 (12%)	0.072	27,837 (6%)	61,325 (7%)	0.042	34,513 (4%)	44,236 (5%)	0.033	36,413 (3%)	32,657 (4%)	0.015
Rheumatoid arthritis	2,666 (1%)	1,204 (1%)	0.013	3,771 (1%)	8,890 (1%)	0.022	3,239 (0%)	4,770 (0%)	0.019	2,706 (0%)	1,281 (0%)	0.024
Stroke	16,690 (7%)	8,542 (9%)	0.073	14,256 (3%)	32,612 (4%)	0.037	10,877 (1%)	12,068 (1%)	0.001	9,400 (1%)	3,384 (0%)	0.063
Venous thromboembolism	8,014 (4%)	4,126 (5%)	0.051	8,172 (2%)	17,846 (2%)	0.021	7,747 (1%)	8,920 (1%)	0.005	6,632 (1%)	3,768 (0%)	0.027

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S14: Characteristics of weighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	57,981	57,843		99,838	99,488		276,241	273,593		312,492	313,346	
Age, median [Q25-Q75]	86 [83-89]	86 [82-89]	0.000	74 [71-81]	74 [72-81]	0.006	50 [45-56]	50 [45-56]	0.003	36 [29-43]	36 [29-43]	0.004
Sex: Female, N(%)	35,828 (62%)	35,895 (62%)	0.005	58,343 (58%)	57,844 (58%)	0.006	130,375 (47%)	129,701 (47%)	0.004	145,270 (46%)	144,384 (46%)	0.008
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.002	15 [15-15]	15 [15-15]	0.005	15 [15-15]	15 [15-15]	0.001	15 [13-16]	15 [14-16]	0.004
Number of GP visits, median [Q25-Q75]	8 [3-15]	8 [4-15]	0.008	4 [0-10]	6 [2-10]	0.013	0 [0-5]	2 [0-6]	0.000	0 [0-4]	0 [0-5]	0.012
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.006	0[0-0]	0[0-0]	0.009	0[0-0]	0[0-0]	0.005	0[0-0]	0[0-0]	0.010
Comorbidities, N(%)**												
Anxiety	11,327 (20%)	11,359 (20%)	0.003	20,281 (20%)	20,181 (20%)	0.001	67,000 (24%)	63,263 (23%)	0.027	65,060 (21%)	64,616 (21%)	0.005
Asthma	3,734 (6%)	3,583 (6%)	0.010	5,213 (5%)	5,179 (5%)	0.001	12,220 (4%)	11,760 (4%)	0.006	15,491 (5%)	15,211 (5%)	0.005
Chronic kidney disease	18,610 (32%)	18,576 (32%)	0.000	15,548 (16%)	15,607 (16%)	0.003	5,583 (2%)	5,399 (2%)	0.003	1,999 (1%)	2,008 (1%)	0.000
COPD	6,980 (12%)	6,907 (12%)	0.003	9,163 (9%)	9,045 (9%)	0.003	6,020 (2%)	6,047 (2%)	0.002	1,998 (1%)	2,000 (1%)	0.000
Dementia	5,678 (10%)	5,771 (10%)	0.006	4,152 (4%)	3,716 (4%)	0.022	1,037 (0%)	980 (0%)	0.003	502 (0%)	506 (0%)	0.000
Depressive disorder	10,920 (19%)	10,923 (19%)	0.001	15,653 (16%)	15,532 (16%)	0.002	26,419 (10%)	24,357 (9%)	0.023	17,262 (6%)	17,507 (6%)	0.003
Diabetes	14,416 (25%)	14,096 (24%)	0.011	20,461 (20%)	20,645 (21%)	0.006	19,302 (7%)	18,543 (7%)	0.008	10,119 (3%)	10,440 (3%)	0.005
GERD	6,784 (12%)	6,826 (12%)	0.003	10,187 (10%)	10,088 (10%)	0.002	12,811 (5%)	12,805 (5%)	0.002	7,750 (2%)	8,224 (3%)	0.009
Heart failure	9,390 (16%)	8,983 (16%)	0.018	7,563 (8%)	7,296 (7%)	0.009	3,044 (1%)	2,699 (1%)	0.011	1,069 (0%)	1,049 (0%)	0.001
Hypertension	37,879 (65%)	37,465 (65%)	0.012	51,262 (51%)	50,608 (51%)	0.010	42,266 (15%)	41,291 (15%)	0.006	15,063 (5%)	15,323 (5%)	0.003
Hypothyroidism	7,310 (13%)	7,166 (12%)	0.007	11,611 (12%)	11,249 (11%)	0.010	16,597 (6%)	16,509 (6%)	0.001	13,155 (4%)	13,617 (4%)	0.007
Malignant neoplastic disease	15,302 (26%)	14,971 (26%)	0.012	19,393 (19%)	18,719 (19%)	0.015	14,311 (5%)	13,981 (5%)	0.003	6,555 (2%)	6,398 (2%)	0.004
Myocardial infarction	2,286 (4%)	2,182 (4%)	0.009	3,130 (3%)	2,924 (3%)	0.011	2,569 (1%)	2,451 (1%)	0.004	875 (0%)	880 (0%)	0.000

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Osteoporosis	9,170 (16%)	9,085 (16%)	0.003	11,182 (11%)	11,201 (11%)	0.002	4,764 (2%)	4,639 (2%)	0.002	1,344 (0%)	1,306 (0%)	0.002
Pneumonia	6,588 (11%)	6,476 (11%)	0.005	7,945 (8%)	7,918 (8%)	0.000	11,759 (4%)	11,273 (4%)	0.007	10,730 (3%)	10,798 (3%)	0.001
Rheumatoid arthritis	755 (1%)	718 (1%)	0.006	1,064 (1%)	1,111 (1%)	0.005	1,113 (0%)	1,026 (0%)	0.004	536 (0%)	461 (0%)	0.006
Stroke	5,070 (9%)	5,006 (9%)	0.003	5,363 (5%)	5,086 (5%)	0.012	3,121 (1%)	3,043 (1%)	0.002	1,199 (0%)	1,253 (0%)	0.003
Venous thromboembolism	2,452 (4%)	2,378 (4%)	0.006	2,660 (3%)	2,526 (3%)	0.008	2,527 (1%)	2,211 (1%)	0.012	1,394 (0%)	1,327 (0%)	0.003

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S15: Characteristics of unweighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	223,960	88,896		433,111	445,581		869,109	706,435		1,068,043	580,329	
Age, median [Q25-Q75]	85 [82-88]	87 [84-90]	0.258	67 [62-72]	78 [74-83]	0.943	48 [43-57]	53 [48-57]	0.127	38 [28-51]	38 [31-43]	0.231
Sex: Female, N(%)	136,891 (61%)	55,704 (63%)	0.032	237,440 (55%)	254,378 (57%)	0.046	409,076 (47%)	341,289 (48%)	0.025	491,528 (46%)	274,150 (47%)	0.024
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.051	15 [15-15]	15 [15-15]	0.206	15 [15-15]	15 [15-15]	0.244	15 [10-16]	15 [15-16]	0.211
Number of GP visits, median [Q25-Q75]	7 [2-13]	10 [5-18]	0.253	3 [0-8]	7 [3-12]	0.247	1 [0-5]	2 [0-7]	0.093	0 [0-4]	2 [0-6]	0.059
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-1]	0.067	0[0-0]	0[0-1]	0.019	0[0-0]	0[0-1]	0.074	0[0-0]	0[0-1]	0.121
Comorbidities, N(%)**												
Anxiety	41,914 (19%)	17,962 (20%)	0.038	97,901 (23%)	91,620 (21%)	0.050	197,933 (23%)	172,434 (24%)	0.039	204,108 (19%)	125,466 (22%)	0.062
Asthma	13,370 (6%)	5,796 (7%)	0.023	19,824 (5%)	25,291 (6%)	0.050	36,788 (4%)	31,586 (4%)	0.012	45,747 (4%)	30,865 (5%)	0.048
Chronic kidney disease	63,046 (28%)	30,745 (35%)	0.139	32,447 (7%)	83,968 (19%)	0.341	22,494 (3%)	13,920 (2%)	0.041	20,416 (2%)	3,171 (1%)	0.124
COPD	24,471 (11%)	11,169 (13%)	0.051	28,773 (7%)	44,207 (10%)	0.119	19,028 (2%)	17,815 (3%)	0.022	15,424 (1%)	3,218 (1%)	0.090
Dementia	16,748 (7%)	10,393 (12%)	0.143	7,389 (2%)	17,606 (4%)	0.136	6,003 (1%)	2,033 (0%)	0.058	5,878 (1%)	856 (0%)	0.068
Depressive disorder	38,161 (17%)	17,894 (20%)	0.079	61,309 (14%)	72,447 (16%)	0.059	77,050 (9%)	69,517 (10%)	0.033	67,070 (6%)	32,476 (6%)	0.029
Diabetes	51,948 (23%)	22,682 (26%)	0.054	65,583 (15%)	99,692 (22%)	0.186	60,242 (7%)	53,330 (8%)	0.024	49,433 (5%)	19,502 (3%)	0.065
GERD	25,370 (11%)	10,611 (12%)	0.019	34,704 (8%)	53,110 (12%)	0.131	37,842 (4%)	37,707 (5%)	0.046	31,662 (3%)	15,544 (3%)	0.017
Heart failure	28,525 (13%)	16,041 (18%)	0.147	16,686 (4%)	34,932 (8%)	0.171	11,920 (1%)	7,057 (1%)	0.034	10,734 (1%)	1,642 (0%)	0.090
Hypertension	138,655 (62%)	59,414 (67%)	0.103	157,291 (36%)	252,365 (57%)	0.416	125,235 (14%)	128,859 (18%)	0.104	97,944 (9%)	26,383 (5%)	0.184

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Hypothyroidism	26,868 (12%)	11,363 (13%)	0.024	42,216 (10%)	53,488 (12%)	0.073	50,384 (6%)	46,214 (7%)	0.031	49,424 (5%)	25,961 (4%)	0.007
Malignant neoplastic disease	54,729 (24%)	23,784 (27%)	0.053	81,399 (19%)	96,204 (22%)	0.070	48,967 (6%)	40,510 (6%)	0.004	37,874 (4%)	11,535 (2%)	0.095
Myocardial infarction	7,890 (4%)	3,590 (4%)	0.027	9,892 (2%)	14,140 (3%)	0.055	7,573 (1%)	7,753 (1%)	0.023	6,182 (1%)	1,405 (0%)	0.053
Osteoporosis	32,250 (14%)	14,701 (17%)	0.059	29,528 (7%)	56,885 (13%)	0.201	16,385 (2%)	14,126 (2%)	0.008	14,380 (1%)	1,876 (0%)	0.113
Pneumonia	22,249 (10%)	10,811 (12%)	0.071	27,831 (6%)	38,127 (9%)	0.081	34,511 (4%)	31,972 (5%)	0.028	36,645 (3%)	21,355 (4%)	0.013
Rheumatoid arthritis	2,666 (1%)	1,189 (1%)	0.013	3,769 (1%)	5,143 (1%)	0.028	3,242 (0%)	2,843 (0%)	0.005	2,700 (0%)	812 (0%)	0.026
Stroke	16,693 (7%)	8,475 (10%)	0.075	14,244 (3%)	24,307 (5%)	0.106	10,868 (1%)	8,114 (1%)	0.009	9,394 (1%)	2,033 (0%)	0.068
Venous thromboembolism	8,023 (4%)	4,072 (5%)	0.050	8,162 (2%)	12,564 (3%)	0.062	7,738 (1%)	6,066 (1%)	0.003	6,658 (1%)	2,434 (0%)	0.028

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S16: Characteristics of weighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 2			Cohort 3		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	120,307	120,802		44,033	43,901	
Age, median [Q25-Q75]	64 [61-66]	64 [61-66]	0.000	64 [61-66]	64 [61-67]	0.002
Sex: Female, N(%)	62,434 (52%)	62,425 (52%)	0.004	23,009 (52%)	22,980 (52%)	0.002
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.003	15 [15-15]	15 [15-15]	0.005
Number of GP visits, median [Q25-Q75]	2 [0-7]	4 [1-8]	0.017	2 [0-7]	2 [0-7]	0.003
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.013	0[0-0]	0[0-0]	0.005
Comorbidities, N(%)**						
Anxiety	28,213 (23%)	27,989 (23%)	0.007	10,333 (23%)	9,717 (22%)	0.032
Asthma	4,909 (4%)	4,785 (4%)	0.006	1,664 (4%)	1,639 (4%)	0.002
Chronic kidney disease	4,390 (4%)	4,526 (4%)	0.005	1,556 (4%)	1,597 (4%)	0.006
COPD	6,702 (6%)	6,846 (6%)	0.004	2,405 (5%)	2,350 (5%)	0.005
Dementia	470 (0%)	397 (0%)	0.010	170 (0%)	171 (0%)	0.000
Depressive disorder	16,483 (14%)	16,258 (13%)	0.007	5,940 (13%)	5,813 (13%)	0.007
Diabetes	16,127 (13%)	16,418 (14%)	0.005	5,698 (13%)	5,705 (13%)	0.002
GERD	9,580 (8%)	9,569 (8%)	0.002	3,206 (7%)	3,216 (7%)	0.002
Heart failure	2,268 (2%)	2,236 (2%)	0.003	844 (2%)	847 (2%)	0.001
Hypertension	39,422 (33%)	39,551 (33%)	0.001	13,589 (31%)	13,737 (31%)	0.009
Hypothyroidism	10,965 (9%)	11,173 (9%)	0.005	4,002 (9%)	3,830 (9%)	0.013
Malignant neoplastic disease	12,621 (10%)	12,418 (10%)	0.007	4,304 (10%)	3,910 (9%)	0.030
Myocardial infarction	2,553 (2%)	2,500 (2%)	0.004	888 (2%)	868 (2%)	0.003
Osteoporosis	6,687 (6%)	6,679 (6%)	0.001	2,155 (5%)	2,200 (5%)	0.005
Pneumonia	6,452 (5%)	6,392 (5%)	0.003	2,243 (5%)	2,167 (5%)	0.007
Rheumatoid arthritis	1,009 (1%)	931 (1%)	0.008	337 (1%)	259 (1%)	0.021
Stroke	2,693 (2%)	2,582 (2%)	0.007	965 (2%)	1,000 (2%)	0.006
Venous thromboembolism	1,686 (1%)	1,469 (1%)	0.016	659 (1%)	541 (1%)	0.023

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S17: Characteristics of unweighted populations in SIDIAP, database, stratified by staggered cohort and exposure status. Exposure is ChAdOx1 vaccine.

	Cohort 2			Cohort 3		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	433,636	323,204		873,400	84,204	
Age, median [Q25-Q75]	67 [62-72]	64 [62-67]	0.241	48 [43-57]	64 [61-67]	0.986
Sex: Female, N(%)	237,722 (55%)	162,269 (50%)	0.093	411,107 (47%)	44,374 (53%)	0.113
Years of prior history*, median [Q25-Q75]	15 [15-15]	15 [15-15]	0.158	15 [15-15]	15 [15-15]	0.281
Number of GP visits, median [Q25-Q75]	3 [0-8]	4 [1-9]	0.037	1 [0-5]	4 [1-8]	0.182
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-1]	0.047	0[0-0]	0[0-0]	0.029
Comorbidities, N(%)**						
Anxiety	97,950 (23%)	77,793 (24%)	0.035	198,282 (23%)	19,626 (23%)	0.014
Asthma	19,861 (5%)	13,894 (4%)	0.014	36,915 (4%)	3,283 (4%)	0.017
Chronic kidney disease	32,497 (7%)	12,206 (4%)	0.162	22,570 (3%)	3,199 (4%)	0.069
COPD	28,826 (7%)	18,658 (6%)	0.036	19,103 (2%)	4,996 (6%)	0.191
Dementia	7,407 (2%)	1,009 (0%)	0.140	6,041 (1%)	345 (0%)	0.038
Depressive disorder	61,377 (14%)	44,463 (14%)	0.011	77,249 (9%)	11,874 (14%)	0.166
Diabetes	65,666 (15%)	45,453 (14%)	0.031	60,383 (7%)	12,142 (14%)	0.245
GERD	34,755 (8%)	28,610 (9%)	0.030	37,840 (4%)	6,754 (8%)	0.154
Heart failure	16,741 (4%)	5,636 (2%)	0.129	11,981 (1%)	1,703 (2%)	0.050
Hypertension	157,484 (36%)	114,179 (35%)	0.021	125,527 (14%)	28,314 (34%)	0.463
Hypothyroidism	42,257 (10%)	30,350 (9%)	0.012	50,476 (6%)	7,763 (9%)	0.131
Malignant neoplastic disease	81,510 (19%)	36,352 (11%)	0.212	49,066 (6%)	7,842 (9%)	0.141
Myocardial infarction	9,910 (2%)	7,242 (2%)	0.003	7,561 (1%)	1,784 (2%)	0.103
Osteoporosis	29,546 (7%)	19,864 (6%)	0.027	16,434 (2%)	4,382 (5%)	0.180
Pneumonia	27,865 (6%)	18,329 (6%)	0.032	34,617 (4%)	4,445 (5%)	0.063
Rheumatoid arthritis	3,762 (1%)	2,557 (1%)	0.008	3,246 (0%)	535 (1%)	0.037
Stroke	14,284 (3%)	6,369 (2%)	0.083	10,884 (1%)	1,915 (2%)	0.078
Venous thromboembolism	8,163 (2%)	3,613 (1%)	0.063	7,746 (1%)	1,110 (1%)	0.041

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S18: Characteristics of weighted populations in CORIVA, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	10,630	10,630		3,863	3,863		17,929	17,929		18,740	18,740	
Age, median [Q25-Q75]	78 [72-83]	78 [72-83]	0.000	70 [65-75]	70 [65-75]	0.000	52 [45-59]	50 [45-59]	0.000	40 [30-51]	40 [30-51]	0.000
Sex: Female, N(%)	7,165 (67%)	7,165 (67%)	0.000	2,439 (63%)	2,439 (63%)	0.000	9,054 (50%)	9,054 (50%)	0.000	8,493 (45%)	8,493 (45%)	0.000
Years of prior history*, median [Q25-Q75]	4 [4-4]	4 [4-4]	0.000	4 [4-4]	4 [4-4]	0.000	4 [4-4]	4 [4-4]	0.000	5 [4-5]	5 [4-5]	0.000
Number of GP visits, median [Q25-Q75]	14 [4-24]	14 [6-23]	0.004	12 [3-23]	12 [5-22]	0.000	2 [0-11]	4 [1-10]	0.002	2 [0-8]	2 [0-8]	0.005
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.002	0[0-0]	0[0-0]	0.000	0[0-0]	0[0-0]	0.002
Comorbidities, N(%)**												
Anxiety	1,060 (10%)	1,076 (10%)	0.005	405 (10%)	415 (11%)	0.008	1,545 (9%)	1,500 (8%)	0.009	1,552 (8%)	1,495 (8%)	0.011
Asthma	996 (9%)	917 (9%)	0.026	372 (10%)	365 (9%)	0.006	936 (5%)	894 (5%)	0.011	845 (5%)	782 (4%)	0.016
Chronic kidney disease	1,015 (10%)	999 (9%)	0.005	336 (9%)	309 (8%)	0.025	347 (2%)	339 (2%)	0.003	212 (1%)	194 (1%)	0.009
COPD	757 (7%)	723 (7%)	0.012	264 (7%)	258 (7%)	0.006	442 (2%)	417 (2%)	0.009	318 (2%)	249 (1%)	0.030
Dementia	438 (4%)	412 (4%)	0.013	106 (3%)	90 (2%)	0.027	120 (1%)	131 (1%)	0.008	71 (0%)	70 (0%)	0.001
Depressive disorder	1,076 (10%)	1,083 (10%)	0.002	462 (12%)	433 (11%)	0.023	1,787 (10%)	1,764 (10%)	0.004	1,679 (9%)	1,739 (9%)	0.011
Diabetes	1,861 (18%)	1,833 (17%)	0.007	640 (17%)	588 (15%)	0.037	1,012 (6%)	963 (5%)	0.012	814 (4%)	764 (4%)	0.014
GERD	1,416 (13%)	1,458 (14%)	0.011	523 (14%)	531 (14%)	0.006	1,562 (9%)	1,600 (9%)	0.007	1,166 (6%)	1,172 (6%)	0.001
Heart failure	3,898 (37%)	3,839 (36%)	0.011	1,003 (26%)	970 (25%)	0.020	1,348 (8%)	1,324 (7%)	0.005	781 (4%)	737 (4%)	0.012
Hypertension	8,016 (75%)	8,221 (77%)	0.045	2,569 (67%)	2,641 (68%)	0.040	5,521 (31%)	5,643 (31%)	0.015	3,359 (18%)	3,538 (19%)	0.025
Hypothyroidism	1,218 (11%)	1,160 (11%)	0.017	419 (11%)	428 (11%)	0.007	981 (5%)	948 (5%)	0.008	654 (3%)	675 (4%)	0.006
Malignant neoplastic disease	1,655 (16%)	1,732 (16%)	0.020	640 (17%)	659 (17%)	0.013	771 (4%)	782 (4%)	0.003	455 (2%)	453 (2%)	0.001
Myocardial infarction	269 (3%)	264 (2%)	0.003	86 (2%)	71 (2%)	0.026	127 (1%)	116 (1%)	0.008	81 (0%)	75 (0%)	0.005
Osteoporosis	675 (6%)	685 (6%)	0.004	187 (5%)	176 (5%)	0.013	233 (1%)	230 (1%)	0.001	131 (1%)	146 (1%)	0.009
Pneumonia	771 (7%)	768 (7%)	0.001	249 (6%)	244 (6%)	0.005	657 (4%)	670 (4%)	0.004	567 (3%)	562 (3%)	0.002
Rheumatoid arthritis	268 (3%)	273 (3%)	0.003	139 (4%)	122 (3%)	0.024	209 (1%)	187 (1%)	0.012	130 (1%)	116 (1%)	0.009

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Stroke	460 (4%)	461 (4%)	0.001	147 (4%)	144 (4%)	0.004	179 (1%)	189 (1%)	0.006	112 (1%)	116 (1%)	0.003
Venous thromboembolism	469 (4%)	472 (4%)	0.001	171 (4%)	123 (3%)	0.064	306 (2%)	301 (2%)	0.002	219 (1%)	201 (1%)	0.009

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S19: Characteristics of unweighted populations in CORIVA, database, stratified by staggered cohort and exposure status. Exposure is any COVID-19 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	23,982	26,736		34,317	4,572		96,423	24,050		147,545	22,245	
Age, median [Q25-Q75]	79 [73-85]	77 [73-82]	0.127	72 [67-81]	70 [66-75]	0.177	54 [46-66]	51 [45-59]	0.227	42 [32-58]	40 [30-51]	0.186
Sex: Female, N(%)	16,532 (69%)	17,342 (65%)	0.087	21,862 (64%)	2,891 (63%)	0.010	48,984 (51%)	12,233 (51%)	0.001	72,057 (49%)	10,026 (45%)	0.076
Years of prior history*, median [Q25-Q75]	4 [4-4]	4 [4-4]	0.019	4 [4-4]	4 [4-4]	0.002	4 [4-4]	4 [4-4]	0.002	5 [4-5]	5 [4-5]	0.003
Number of GP visits, median [Q25-Q75]	11 [1-22]	16 [8-26]	0.216	9 [1-20]	13 [6-23]	0.147	2 [0-10]	5 [1-11]	0.088	1 [0-6]	3 [1-8]	0.106
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.029	0[0-0]	0[0-0]	0.027	0[0-0]	0[0-0]	0.008	0[0-0]	0[0-0]	0.035
Comorbidities, N(%)**												
Anxiety	1,966 (8%)	3,238 (12%)	0.130	2,817 (8%)	513 (11%)	0.102	6,406 (7%)	2,162 (9%)	0.087	9,287 (6%)	1,847 (8%)	0.077
Asthma	1,986 (8%)	2,668 (10%)	0.059	2,653 (8%)	447 (10%)	0.072	4,385 (5%)	1,250 (5%)	0.030	5,405 (4%)	968 (4%)	0.035
Chronic kidney disease	2,270 (9%)	2,507 (9%)	0.003	2,939 (9%)	367 (8%)	0.019	2,523 (3%)	415 (2%)	0.061	2,335 (2%)	221 (1%)	0.052
COPD	1,569 (7%)	1,976 (7%)	0.033	2,015 (6%)	312 (7%)	0.039	2,743 (3%)	529 (2%)	0.041	2,558 (2%)	294 (1%)	0.034
Dementia	1,152 (5%)	819 (3%)	0.090	1,297 (4%)	100 (2%)	0.094	1,147 (1%)	151 (1%)	0.059	1,067 (1%)	76 (0%)	0.052
Depressive disorder	2,066 (9%)	3,233 (12%)	0.114	3,189 (9%)	535 (12%)	0.079	7,286 (8%)	2,561 (11%)	0.108	10,034 (7%)	2,152 (10%)	0.105
Diabetes	3,971 (17%)	4,734 (18%)	0.030	4,818 (14%)	712 (16%)	0.043	6,065 (6%)	1,228 (5%)	0.051	6,594 (4%)	901 (4%)	0.021
GERD	2,533 (11%)	4,585 (17%)	0.192	3,364 (10%)	667 (15%)	0.147	6,287 (7%)	2,322 (10%)	0.115	7,455 (5%)	1,427 (6%)	0.059
Heart failure	8,724 (36%)	9,642 (36%)	0.007	9,570 (28%)	1,139 (25%)	0.068	9,982 (10%)	1,607 (7%)	0.132	9,182 (6%)	828 (4%)	0.115
Hypertension	16,651 (69%)	21,677 (81%)	0.272	20,654 (60%)	3,167 (69%)	0.191	29,974 (31%)	7,555 (31%)	0.007	27,992 (19%)	4,172 (19%)	0.006
Hypothyroidism	2,496 (10%)	3,094 (12%)	0.037	3,198 (9%)	515 (11%)	0.064	4,725 (5%)	1,309 (5%)	0.025	5,180 (4%)	799 (4%)	0.004
Malignant neoplastic disease	3,194 (13%)	5,044 (19%)	0.151	5,061 (15%)	782 (17%)	0.064	4,587 (5%)	1,011 (4%)	0.027	4,134 (3%)	523 (2%)	0.028
Myocardial infarction	548 (2%)	743 (3%)	0.031	696 (2%)	86 (2%)	0.011	840 (1%)	146 (1%)	0.031	777 (1%)	87 (0%)	0.020
Osteoporosis	1,273 (5%)	2,015 (8%)	0.091	1,446 (4%)	212 (5%)	0.021	1,545 (2%)	289 (1%)	0.034	1,408 (1%)	164 (1%)	0.024
Pneumonia	1,611 (7%)	2,046 (8%)	0.036	2,081 (6%)	291 (6%)	0.012	3,342 (3%)	898 (4%)	0.014	3,919 (3%)	683 (3%)	0.025

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Rheumatoid arthritis	493 (2%)	817 (3%)	0.063	891 (3%)	153 (3%)	0.044	1,022 (1%)	250 (1%)	0.002	987 (1%)	137 (1%)	0.007
Stroke	1,137 (5%)	1,011 (4%)	0.048	1,450 (4%)	169 (4%)	0.027	1,359 (1%)	232 (1%)	0.041	1,256 (1%)	133 (1%)	0.030
Venous thromboembolism	1,058 (4%)	1,207 (5%)	0.005	1,349 (4%)	149 (3%)	0.036	1,808 (2%)	387 (2%)	0.020	1,788 (1%)	235 (1%)	0.015

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S20: Characteristics of weighted populations in CORIVA, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	9,118	9,118		3,465	3,465		14,414	14,414		13,759	13,759	
Age, median [Q25-Q75]	78 [73-83]	78 [73-83]	0.000	70 [65-75]	70 [65-75]	0.000	50 [45-59]	50 [45-59]	0.000	38 [30-50]	38 [30-50]	0.000
Sex: Female, N(%)	6,206 (68%)	6,206 (68%)	0.000	2,197 (63%)	2,197 (63%)	0.000	7,324 (51%)	7,324 (51%)	0.000	6,552 (48%)	6,552 (48%)	0.000
Years of prior history*, median [Q25-Q75]	4 [4-4]	4 [4-4]	0.000	4 [4-4]	4 [4-4]	0.000	4 [4-4]	4 [4-4]	0.000	5 [4-5]	5 [4-5]	0.000
Number of GP visits, median [Q25-Q75]	14 [4-25]	14 [7-24]	0.004	12 [3-23]	12 [5-22]	0.000	2 [0-10]	4 [1-10]	0.002	2 [0-8]	2 [0-8]	0.004
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.002	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.000	0[0-0]	0[0-0]	0.001
Comorbidities, N(%)**												
Anxiety	935 (10%)	956 (10%)	0.007	367 (11%)	373 (11%)	0.006	1,284 (9%)	1,278 (9%)	0.001	1,206 (9%)	1,170 (9%)	0.009
Asthma	891 (10%)	822 (9%)	0.026	340 (10%)	333 (10%)	0.007	747 (5%)	718 (5%)	0.009	644 (5%)	615 (4%)	0.010
Chronic kidney disease	939 (10%)	905 (10%)	0.012	302 (9%)	278 (8%)	0.025	225 (2%)	220 (2%)	0.003	144 (1%)	132 (1%)	0.008
COPD	666 (7%)	639 (7%)	0.011	238 (7%)	227 (7%)	0.013	333 (2%)	316 (2%)	0.008	223 (2%)	171 (1%)	0.032
Dementia	403 (4%)	377 (4%)	0.014	91 (3%)	81 (2%)	0.018	75 (1%)	72 (0%)	0.002	46 (0%)	38 (0%)	0.012
Depressive disorder	960 (11%)	966 (11%)	0.002	420 (12%)	411 (12%)	0.008	1,472 (10%)	1,485 (10%)	0.003	1,311 (10%)	1,367 (10%)	0.014
Diabetes	1,647 (18%)	1,624 (18%)	0.007	576 (17%)	531 (15%)	0.036	747 (5%)	731 (5%)	0.005	596 (4%)	565 (4%)	0.011
GERD	1,240 (14%)	1,268 (14%)	0.009	476 (14%)	473 (14%)	0.003	1,283 (9%)	1,322 (9%)	0.009	892 (6%)	900 (7%)	0.002
Heart failure	3,527 (39%)	3,470 (38%)	0.013	911 (26%)	877 (25%)	0.022	920 (6%)	899 (6%)	0.006	519 (4%)	484 (4%)	0.014
Hypertension	7,040 (77%)	7,234 (79%)	0.051	2,327 (67%)	2,384 (69%)	0.036	4,296 (30%)	4,340 (30%)	0.007	2,391 (17%)	2,479 (18%)	0.017
Hypothyroidism	1,080 (12%)	1,035 (11%)	0.015	376 (11%)	388 (11%)	0.011	770 (5%)	743 (5%)	0.009	500 (4%)	503 (4%)	0.001
Malignant neoplastic disease	1,485 (16%)	1,565 (17%)	0.023	579 (17%)	594 (17%)	0.012	582 (4%)	606 (4%)	0.008	324 (2%)	317 (2%)	0.003
Myocardial infarction	248 (3%)	234 (3%)	0.010	78 (2%)	68 (2%)	0.020	97 (1%)	88 (1%)	0.008	52 (0%)	52 (0%)	0.000
Osteoporosis	615 (7%)	632 (7%)	0.007	170 (5%)	167 (5%)	0.003	167 (1%)	161 (1%)	0.004	97 (1%)	107 (1%)	0.008
Pneumonia	698 (8%)	696 (8%)	0.001	222 (6%)	222 (6%)	0.001	543 (4%)	505 (4%)	0.014	418 (3%)	429 (3%)	0.005
Rheumatoid arthritis	240 (3%)	239 (3%)	0.001	128 (4%)	110 (3%)	0.028	167 (1%)	148 (1%)	0.013	97 (1%)	88 (1%)	0.008
Stroke	419 (5%)	417 (5%)	0.001	134 (4%)	131 (4%)	0.004	121 (1%)	130 (1%)	0.007	74 (1%)	82 (1%)	0.008

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Venous thromboembolism	438 (5%)	416 (5%)	0.011	153 (4%)	117 (3%)	0.053	220 (2%)	214 (1%)	0.003	159 (1%)	138 (1%)	0.015

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S21: Characteristics of unweighted populations in CORIVA, database, stratified by staggered cohort and exposure status. Exposure is BNT162b2 vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
N (individuals)	24,073	19,686		34,320	4,067		96,471	18,645		147,553	15,683	
Age, median [Q25-Q75]	79 [73-85]	79 [74-83]	0.040	72 [67-81]	70 [66-75]	0.174	54 [46-66]	51 [45-59]	0.257	42 [32-58]	38 [30-50]	0.216
Sex: Female, N(%)	16,582 (69%)	13,022 (66%)	0.058	21,867 (64%)	2,582 (63%)	0.005	49,023 (51%)	9,570 (51%)	0.010	72,056 (49%)	7,500 (48%)	0.020
Years of prior history*, median [Q25-Q75]	4 [4-4]	4 [4-4]	0.026	4 [4-4]	4 [4-4]	0.000	4 [4-4]	4 [4-4]	0.000	5 [4-5]	5 [4-5]	0.003
Number of GP visits, median [Q25-Q75]	11 [1-22]	17 [9-27]	0.252	9 [1-20]	13 [6-23]	0.150	2 [0-10]	5 [1-11]	0.077	1 [0-6]	4 [1-9]	0.116
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.021	0[0-0]	0[0-0]	0.038	0[0-0]	0[0-0]	0.020	0[0-0]	0[0-0]	0.037
Comorbidities, N(%)**												
Anxiety	1,966 (8%)	2,429 (12%)	0.138	2,816 (8%)	458 (11%)	0.103	6,416 (7%)	1,780 (10%)	0.106	9,291 (6%)	1,384 (9%)	0.096
Asthma	2,004 (8%)	2,029 (10%)	0.068	2,653 (8%)	404 (10%)	0.078	4,393 (5%)	969 (5%)	0.030	5,403 (4%)	730 (5%)	0.050
Chronic kidney disease	2,284 (9%)	2,041 (10%)	0.029	2,943 (9%)	326 (8%)	0.020	2,537 (3%)	263 (1%)	0.087	2,339 (2%)	146 (1%)	0.059
COPD	1,587 (7%)	1,501 (8%)	0.040	2,013 (6%)	274 (7%)	0.036	2,746 (3%)	388 (2%)	0.049	2,560 (2%)	193 (1%)	0.042
Dementia	1,162 (5%)	688 (3%)	0.067	1,301 (4%)	90 (2%)	0.093	1,136 (1%)	81 (0%)	0.083	1,062 (1%)	40 (0%)	0.067
Depressive disorder	2,070 (9%)	2,449 (12%)	0.125	3,188 (9%)	502 (12%)	0.098	7,303 (8%)	2,075 (11%)	0.122	10,051 (7%)	1,623 (10%)	0.127
Diabetes	3,964 (16%)	3,599 (18%)	0.048	4,814 (14%)	638 (16%)	0.047	6,071 (6%)	900 (5%)	0.064	6,594 (4%)	644 (4%)	0.018
GERD	2,532 (11%)	3,307 (17%)	0.184	3,364 (10%)	587 (14%)	0.142	6,296 (7%)	1,858 (10%)	0.125	7,446 (5%)	1,050 (7%)	0.070
Heart failure	8,750 (36%)	7,743 (39%)	0.062	9,570 (28%)	1,026 (25%)	0.060	10,000 (10%)	1,057 (6%)	0.174	9,185 (6%)	525 (3%)	0.135
Hypertension	16,718 (69%)	16,325 (83%)	0.321	20,659 (60%)	2,838 (70%)	0.202	29,995 (31%)	5,624 (30%)	0.020	27,979 (19%)	2,798 (18%)	0.029
Hypothyroidism	2,526 (10%)	2,352 (12%)	0.046	3,197 (9%)	464 (11%)	0.069	4,732 (5%)	988 (5%)	0.018	5,173 (4%)	576 (4%)	0.009
Malignant neoplastic disease	3,216 (13%)	3,873 (20%)	0.171	5,060 (15%)	700 (17%)	0.067	4,587 (5%)	764 (4%)	0.032	4,139 (3%)	352 (2%)	0.036
Myocardial infarction	539 (2%)	591 (3%)	0.048	696 (2%)	82 (2%)	0.001	850 (1%)	108 (1%)	0.035	779 (1%)	58 (0%)	0.024
Osteoporosis	1,283 (5%)	1,628 (8%)	0.117	1,447 (4%)	200 (5%)	0.034	1,550 (2%)	197 (1%)	0.048	1,403 (1%)	117 (1%)	0.022
Pneumonia	1,621 (7%)	1,624 (8%)	0.058	2,080 (6%)	264 (6%)	0.018	3,353 (3%)	672 (4%)	0.007	3,920 (3%)	499 (3%)	0.031

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	Unvaccinated	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**	**Unvaccinated**	**Vaccinated**	**ASMD**
Rheumatoid arthritis	491 (2%)	603 (3%)	0.065	892 (3%)	137 (3%)	0.045	1,025 (1%)	190 (1%)	0.004	990 (1%)	99 (1%)	0.005
Stroke	1,129 (5%)	834 (4%)	0.022	1,447 (4%)	152 (4%)	0.025	1,370 (1%)	155 (1%)	0.056	1,254 (1%)	90 (1%)	0.033
Venous thromboembolism	1,067 (4%)	972 (5%)	0.024	1,353 (4%)	140 (3%)	0.027	1,808 (2%)	266 (1%)	0.035	1,785 (1%)	156 (1%)	0.021

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S22: Characteristics of weighted populations in CPRD AURUM database, stratified by staggered cohort and vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**
N (individuals)	48,138	48,432		306,114	307,972		35,517	35,840		117,220	116,722	
Age, median [Q25-Q75]	80 [77-85]	80 [77-85]	0.003	66 [57-72]	66 [57-72]	0.003	54 [46-60]	54 [46-60]	0.000	42 [39-46]	42 [39-46]	0.004
Sex: Female, N(%)	27,920 (58%)	27,962 (58%)	0.005	167,623 (55%)	168,473 (55%)	0.001	21,299 (60%)	21,484 (60%)	0.000	54,350 (46%)	54,304 (47%)	0.003
Years of prior history*, median [Q25-Q75]	24 [9-36]	25 [11-36]	0.011	21 [9-32]	21 [9-32]	0.000	17 [8-27]	17 [7-27]	0.007	9 [4-17]	9 [4-17]	0.001
Number of GP visits, median [Q25-Q75]	12 [6-19]	12 [6-19]	0.003	10 [5-16]	10 [6-16]	0.001	8 [4-14]	8 [4-14]	0.012	4 [1-8]	4 [1-8]	0.008
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.006	0[0-0]	0[0-0]	0.003	0[0-0]	0[0-0]	0.013	0[0-0]	0[0-0]	0.003
Comorbidities, N(%)**												
Anxiety	7,101 (15%)	7,442 (15%)	0.017	62,230 (20%)	61,892 (20%)	0.006	7,419 (21%)	7,362 (21%)	0.009	19,086 (16%)	19,543 (17%)	0.012
Asthma	5,241 (11%)	5,396 (11%)	0.008	57,043 (19%)	56,551 (18%)	0.007	6,365 (18%)	6,097 (17%)	0.024	9,186 (8%)	9,059 (8%)	0.003
Chronic kidney disease	11,403 (24%)	11,809 (24%)	0.016	28,219 (9%)	29,790 (10%)	0.016	1,565 (4%)	1,700 (5%)	0.016	793 (1%)	897 (1%)	0.011
COPD	4,080 (8%)	4,014 (8%)	0.007	20,765 (7%)	20,456 (7%)	0.006	661 (2%)	669 (2%)	0.000	424 (0%)	471 (0%)	0.007
Dementia	3,667 (8%)	3,510 (7%)	0.014	2,733 (1%)	2,137 (1%)	0.022	419 (1%)	288 (1%)	0.038	109 (0%)	60 (0%)	0.016
Depressive disorder	5,666 (12%)	5,885 (12%)	0.012	56,642 (19%)	57,377 (19%)	0.003	6,508 (18%)	6,556 (18%)	0.001	16,027 (14%)	16,191 (14%)	0.006
Diabetes	8,849 (18%)	9,020 (19%)	0.006	50,019 (16%)	50,218 (16%)	0.001	2,560 (7%)	2,850 (8%)	0.028	3,231 (3%)	3,106 (3%)	0.006
GERD	2,637 (5%)	2,694 (6%)	0.004	16,666 (5%)	16,658 (5%)	0.002	1,479 (4%)	1,443 (4%)	0.007	3,087 (3%)	3,046 (3%)	0.001
Heart failure	2,843 (6%)	2,904 (6%)	0.004	6,822 (2%)	6,936 (2%)	0.002	443 (1%)	361 (1%)	0.023	204 (0%)	202 (0%)	0.000
Hypertension	25,422 (53%)	26,106 (54%)	0.022	103,545 (34%)	105,521 (34%)	0.009	6,295 (18%)	6,514 (18%)	0.012	6,692 (6%)	6,522 (6%)	0.005
Hypothyroidism	4,806 (10%)	4,978 (10%)	0.010	22,667 (7%)	23,290 (8%)	0.006	1,845 (5%)	1,886 (5%)	0.003	2,985 (3%)	3,025 (3%)	0.003
Malignant neoplastic disease	10,958 (23%)	11,284 (23%)	0.013	38,507 (13%)	40,499 (13%)	0.017	2,019 (6%)	2,152 (6%)	0.014	1,504 (1%)	1,576 (1%)	0.006
Myocardial infarction	2,481 (5%)	2,516 (5%)	0.002	11,083 (4%)	11,197 (4%)	0.001	425 (1%)	521 (1%)	0.022	256 (0%)	349 (0%)	0.016
Osteoporosis	5,117 (11%)	5,335 (11%)	0.012	11,759 (4%)	11,858 (4%)	0.000	719 (2%)	667 (2%)	0.012	344 (0%)	372 (0%)	0.004

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD
Pneumonia	2,576 (5%)	2,527 (5%)	0.006	9,543 (3%)	9,423 (3%)	0.003	808 (2%)	692 (2%)	0.024	979 (1%)	904 (1%)	0.007
Rheumatoid arthritis	935 (2%)	1,005 (2%)	0.010	6,476 (2%)	6,640 (2%)	0.003	284 (1%)	344 (1%)	0.017	216 (0%)	258 (0%)	0.008
Stroke	2,314 (5%)	2,418 (5%)	0.009	7,284 (2%)	7,949 (3%)	0.013	446 (1%)	414 (1%)	0.009	294 (0%)	336 (0%)	0.007
Venous thromboembolism	3,001 (6%)	3,101 (6%)	0.007	11,833 (4%)	12,315 (4%)	0.007	812 (2%)	853 (2%)	0.006	601 (1%)	765 (1%)	0.019

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S23: Characteristics of unweighted populations in CPRD AURUM database, stratified by staggered cohort and vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**
N (individuals)	219,804	332,790		969,262	594,262		1,473,602	54,102		542,670	1,335,671	
Age, median [Q25-Q75]	80 [77-85]	82 [79-86]	0.129	69 [63-73]	67 [56-73]	0.148	55 [51-60]	58 [50-82]	0.381	45 [42-48]	31 [25-37]	1.319
Sex: Female, N(%)	127,656 (58%)	186,481 (56%)	0.041	528,692 (55%)	324,259 (55%)	0.000	739,444 (50%)	32,310 (60%)	0.193	242,758 (45%)	625,195 (47%)	0.042
Years of prior history*, median [Q25-Q75]	24 [9-36]	26 [13-38]	0.059	21 [9-33]	21 [9-32]	0.028	17 [8-27]	19 [8-30]	0.116	10 [5-18]	7 [3-19]	0.146
Number of GP visits, median [Q25-Q75]	12 [6-19]	11 [6-18]	0.038	10 [6-17]	11 [6-17]	0.015	7 [3-12]	10 [5-17]	0.294	3 [1-8]	3 [1-7]	0.045
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.183	0[0-0]	0[0-0]	0.014	0[0-0]	0[0-0]	0.171	0[0-0]	0[0-0]	0.008
Comorbidities, N(%)**												
Anxiety	33,355 (15%)	49,410 (15%)	0.009	187,469 (19%)	122,087 (21%)	0.030	281,846 (19%)	10,441 (19%)	0.004	86,805 (16%)	218,144 (16%)	0.009
Asthma	24,009 (11%)	37,889 (11%)	0.015	159,162 (16%)	116,596 (20%)	0.083	200,995 (14%)	8,668 (16%)	0.067	41,393 (8%)	114,378 (9%)	0.034
Chronic kidney disease	51,053 (23%)	86,899 (26%)	0.067	97,339 (10%)	56,923 (10%)	0.016	24,198 (2%)	6,692 (12%)	0.430	2,879 (1%)	2,664 (0%)	0.055
COPD	19,105 (9%)	28,317 (9%)	0.007	67,700 (7%)	39,595 (7%)	0.013	13,621 (1%)	2,063 (4%)	0.191	1,510 (0%)	942 (0%)	0.050
Dementia	16,795 (8%)	16,138 (5%)	0.116	12,106 (1%)	3,720 (1%)	0.065	1,896 (0%)	1,323 (2%)	0.207	447 (0%)	119 (0%)	0.034
Depressive disorder	27,009 (12%)	37,831 (11%)	0.028	170,626 (18%)	112,753 (19%)	0.035	256,789 (17%)	9,046 (17%)	0.019	74,210 (14%)	155,037 (12%)	0.062
Diabetes	39,751 (18%)	61,929 (19%)	0.014	154,348 (16%)	98,676 (17%)	0.018	78,394 (5%)	5,817 (11%)	0.201	9,729 (2%)	12,040 (1%)	0.077
GERD	12,156 (6%)	20,204 (6%)	0.023	52,694 (5%)	32,288 (5%)	0.000	56,444 (4%)	2,506 (5%)	0.040	13,807 (3%)	21,599 (2%)	0.065
Heart failure	13,147 (6%)	20,430 (6%)	0.007	24,276 (3%)	13,120 (2%)	0.020	5,673 (0%)	1,529 (3%)	0.195	681 (0%)	448 (0%)	0.033
Hypertension	114,922 (52%)	186,367 (56%)	0.075	345,014 (36%)	200,762 (34%)	0.038	224,543 (15%)	15,536 (29%)	0.330	31,330 (6%)	19,649 (1%)	0.232
Hypothyroidism	21,855 (10%)	34,512 (10%)	0.014	73,992 (8%)	44,456 (7%)	0.006	64,026 (4%)	3,642 (7%)	0.104	13,920 (3%)	18,516 (1%)	0.085
Malignant neoplastic disease	49,023 (22%)	82,610 (25%)	0.059	131,135 (14%)	76,103 (13%)	0.021	55,601 (4%)	6,311 (12%)	0.299	7,505 (1%)	5,104 (0%)	0.107
Myocardial infarction	11,236 (5%)	18,289 (5%)	0.017	33,142 (3%)	23,019 (4%)	0.024	11,237 (1%)	1,464 (3%)	0.149	861 (0%)	715 (0%)	0.032

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD
Osteoporosis	22,986 (10%)	36,535 (11%)	0.017	42,319 (4%)	21,789 (4%)	0.036	12,455 (1%)	2,632 (5%)	0.243	1,380 (0%)	1,028 (0%)	0.044
Pneumonia	12,101 (6%)	16,297 (5%)	0.027	33,170 (3%)	17,567 (3%)	0.027	20,399 (1%)	1,658 (3%)	0.114	4,297 (1%)	7,793 (1%)	0.025
Rheumatoid arthritis	4,544 (2%)	6,693 (2%)	0.004	20,294 (2%)	12,878 (2%)	0.005	8,463 (1%)	667 (1%)	0.070	821 (0%)	891 (0%)	0.026
Stroke	10,876 (5%)	15,601 (5%)	0.012	24,851 (3%)	15,056 (3%)	0.002	9,547 (1%)	1,281 (2%)	0.141	1,153 (0%)	1,022 (0%)	0.036
Venous thromboembolism	13,782 (6%)	21,549 (6%)	0.008	39,415 (4%)	23,632 (4%)	0.005	24,312 (2%)	1,980 (4%)	0.125	1,936 (0%)	2,541 (0%)	0.032

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S24: Characteristics of weighted populations in CPRD GOLD database, stratified by staggered cohort and vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**
N (individuals)	7,490	7,490		77,012	76,978		24,590	24,597		49,538	49,577	
Age, median [Q25-Q75]	82 [78-86]	80 [78-86]	0.000	70 [65-74]	70 [65-74]	0.004	54 [49-59]	54 [49-59]	0.009	42 [37-46]	42 [37-47]	0.003
Sex: Female, N(%)	4,266 (57%)	4,266 (57%)	0.000	41,394 (54%)	41,607 (54%)	0.006	13,052 (53%)	13,078 (53%)	0.002	22,308 (45%)	22,520 (45%)	0.008
Years of prior history*, median [Q25-Q75]	18 [12-21]	18 [11-21]	0.000	17 [12-20]	17 [12-20]	0.005	17 [12-20]	17 [12-20]	0.004	14 [6-18]	14 [6-18]	0.005
Number of GP visits, median [Q25-Q75]	14 [9-21]	14 [9-21]	0.007	10 [6-16]	10 [6-17]	0.012	6 [2-11]	6 [2-11]	0.009	2 [0-6]	2 [0-7]	0.008
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.004	0[0-0]	0[0-0]	0.002	0[0-0]	0[0-0]	0.011	0[0-0]	0[0-0]	0.002
Comorbidities, N(%)**												
Anxiety	1,008 (13%)	1,015 (14%)	0.002	12,765 (17%)	12,897 (17%)	0.005	5,218 (21%)	5,293 (22%)	0.007	8,501 (17%)	8,578 (17%)	0.004
Asthma	783 (10%)	774 (10%)	0.004	9,867 (13%)	10,028 (13%)	0.006	2,993 (12%)	2,826 (11%)	0.021	3,700 (7%)	3,568 (7%)	0.010
Chronic kidney disease	1,711 (23%)	1,694 (23%)	0.005	7,209 (9%)	7,045 (9%)	0.007	530 (2%)	411 (2%)	0.035	193 (0%)	202 (0%)	0.003
COPD	568 (8%)	583 (8%)	0.008	5,739 (7%)	5,725 (7%)	0.001	321 (1%)	274 (1%)	0.017	170 (0%)	165 (0%)	0.002
Dementia	543 (7%)	495 (7%)	0.025	726 (1%)	737 (1%)	0.001	55 (0%)	74 (0%)	0.015	28 (0%)	21 (0%)	0.006
Depressive disorder	733 (10%)	729 (10%)	0.002	11,461 (15%)	11,746 (15%)	0.011	4,478 (18%)	4,427 (18%)	0.005	7,038 (14%)	7,119 (14%)	0.004
Diabetes	1,114 (15%)	1,107 (15%)	0.003	10,260 (13%)	10,130 (13%)	0.005	1,051 (4%)	963 (4%)	0.018	768 (2%)	654 (1%)	0.019
GERD	451 (6%)	447 (6%)	0.002	3,691 (5%)	3,753 (5%)	0.004	763 (3%)	770 (3%)	0.001	975 (2%)	978 (2%)	0.000
Heart failure	395 (5%)	411 (5%)	0.009	1,763 (2%)	1,819 (2%)	0.005	125 (1%)	81 (0%)	0.028	50 (0%)	54 (0%)	0.002
Hypertension	2,761 (37%)	2,754 (37%)	0.002	21,627 (28%)	21,686 (28%)	0.002	3,184 (13%)	3,261 (13%)	0.009	1,842 (4%)	1,975 (4%)	0.014
Hypothyroidism	579 (8%)	566 (8%)	0.007	4,932 (6%)	4,869 (6%)	0.003	1,064 (4%)	1,065 (4%)	0.000	979 (2%)	1,028 (2%)	0.007
Malignant neoplastic disease	1,632 (22%)	1,744 (23%)	0.036	10,682 (14%)	9,986 (13%)	0.026	1,007 (4%)	1,089 (4%)	0.017	598 (1%)	660 (1%)	0.011
Myocardial infarction	330 (4%)	325 (4%)	0.004	2,919 (4%)	2,943 (4%)	0.002	239 (1%)	217 (1%)	0.010	90 (0%)	85 (0%)	0.002

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**
Osteoporosis	665 (9%)	638 (9%)	0.013	3,388 (4%)	3,496 (5%)	0.007	323 (1%)	314 (1%)	0.003	103 (0%)	92 (0%)	0.005
Pneumonia	286 (4%)	258 (3%)	0.020	1,807 (2%)	1,724 (2%)	0.007	263 (1%)	252 (1%)	0.005	271 (1%)	265 (1%)	0.002
Rheumatoid arthritis	111 (1%)	111 (1%)	0.001	1,437 (2%)	1,460 (2%)	0.002	123 (1%)	115 (0%)	0.005	58 (0%)	40 (0%)	0.011
Stroke	325 (4%)	321 (4%)	0.003	2,134 (3%)	2,072 (3%)	0.005	207 (1%)	148 (1%)	0.028	96 (0%)	91 (0%)	0.002
Venous thromboembolism	306 (4%)	341 (5%)	0.023	2,125 (3%)	2,153 (3%)	0.002	288 (1%)	285 (1%)	0.001	146 (0%)	198 (0%)	0.018

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S25: Characteristics of unweighted populations in CPRD GOLD database, stratified by staggered cohort and vaccine.

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**	**ChAdOx1**	**BNT162b2**	**ASMD**
N (individuals)	82,406	32,755		302,999	180,670		423,876	36,748		147,744	365,096	
Age, median [Q25-Q75]	84 [82-88]	81 [79-86]	0.351	71 [66-76]	70 [66-74]	0.132	56 [52-61]	54 [50-60]	0.171	44 [41-48]	31 [24-37]	1.090
Sex: Female, N(%)	47,915 (58%)	18,415 (56%)	0.039	162,753 (54%)	97,747 (54%)	0.008	210,283 (50%)	19,649 (53%)	0.077	64,999 (44%)	167,510 (46%)	0.038
Years of prior history*, median [Q25-Q75]	17 [14-20]	18 [13-21]	0.001	17 [13-19]	17 [12-19]	0.014	17 [10-19]	18 [12-20]	0.107	14 [7-18]	15 [6-18]	0.017
Number of GP visits, median [Q25-Q75]	14 [9-21]	14 [9-21]	0.005	11 [7-18]	10 [6-16]	0.122	6 [2-12]	6 [2-11]	0.069	3 [0-7]	2 [0-6]	0.098
Number of PCR tests, median [Q25-Q75]	0[0-0]	0[0-0]	0.006	0[0-0]	0[0-0]	0.009	0[0-0]	0[0-0]	0.021	0[0-0]	0[0-0]	0.011
Comorbidities, N(%)**												
Anxiety	9,923 (12%)	4,336 (13%)	0.036	49,010 (16%)	28,697 (16%)	0.008	75,258 (18%)	8,004 (22%)	0.101	24,601 (17%)	59,094 (16%)	0.013
Asthma	8,491 (10%)	3,250 (10%)	0.013	39,988 (13%)	21,396 (12%)	0.041	48,034 (11%)	4,053 (11%)	0.010	10,517 (7%)	33,240 (9%)	0.073
Chronic kidney disease	21,564 (26%)	7,430 (23%)	0.081	34,168 (11%)	15,033 (8%)	0.100	7,553 (2%)	566 (2%)	0.019	739 (1%)	578 (0%)	0.060
COPD	6,837 (8%)	2,417 (7%)	0.034	27,472 (9%)	11,545 (6%)	0.100	6,334 (1%)	348 (1%)	0.050	640 (0%)	285 (0%)	0.070
Dementia	4,773 (6%)	1,895 (6%)	0.000	5,124 (2%)	1,375 (1%)	0.085	548 (0%)	87 (0%)	0.025	126 (0%)	29 (0%)	0.036
Depressive disorder	6,998 (8%)	3,310 (10%)	0.056	45,154 (15%)	26,294 (15%)	0.010	70,703 (17%)	6,569 (18%)	0.032	21,711 (15%)	39,181 (11%)	0.119
Diabetes	12,387 (15%)	4,882 (15%)	0.004	43,647 (14%)	22,552 (12%)	0.056	24,219 (6%)	1,191 (3%)	0.120	2,254 (2%)	2,507 (1%)	0.080
GERD	4,054 (5%)	2,012 (6%)	0.054	14,294 (5%)	8,525 (5%)	0.000	14,447 (3%)	1,093 (3%)	0.025	2,883 (2%)	5,213 (1%)	0.041
Heart failure	5,058 (6%)	1,782 (5%)	0.030	9,180 (3%)	3,669 (2%)	0.064	2,308 (1%)	100 (0%)	0.043	191 (0%)	98 (0%)	0.037
Hypertension	31,217 (38%)	11,995 (37%)	0.026	88,833 (29%)	49,763 (28%)	0.039	59,554 (14%)	4,679 (13%)	0.039	6,761 (5%)	4,776 (1%)	0.194
Hypothyroidism	7,042 (9%)	2,373 (7%)	0.048	20,521 (7%)	11,106 (6%)	0.025	17,382 (4%)	1,553 (4%)	0.006	3,245 (2%)	4,180 (1%)	0.082
Malignant neoplastic disease	18,950 (23%)	6,972 (21%)	0.041	42,687 (14%)	22,617 (13%)	0.046	17,903 (4%)	1,522 (4%)	0.004	2,192 (1%)	1,796 (0%)	0.100
Myocardial infarction	4,500 (5%)	1,603 (5%)	0.026	13,456 (4%)	6,621 (4%)	0.039	5,280 (1%)	271 (1%)	0.051	330 (0%)	154 (0%)	0.050

	Cohort 1			Cohort 2			Cohort 3			Cohort 4		
	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD	ChAdOx1	BNT162b2	ASMD
Osteoporosis	8,564 (10%)	2,770 (8%)	0.066	16,117 (5%)	7,531 (4%)	0.054	4,330 (1%)	458 (1%)	0.021	408 (0%)	205 (0%)	0.054
Pneumonia	3,231 (4%)	1,032 (3%)	0.042	8,604 (3%)	3,418 (2%)	0.062	4,527 (1%)	345 (1%)	0.013	921 (1%)	1,696 (0%)	0.022
Rheumatoid arthritis	1,257 (2%)	512 (2%)	0.003	5,999 (2%)	2,929 (2%)	0.027	2,405 (1%)	154 (0%)	0.021	191 (0%)	111 (0%)	0.035
Stroke	4,193 (5%)	1,490 (5%)	0.025	10,213 (3%)	4,632 (3%)	0.048	3,789 (1%)	199 (1%)	0.042	369 (0%)	225 (0%)	0.048
Venous thromboembolism	3,550 (4%)	1,389 (4%)	0.003	9,466 (3%)	4,490 (2%)	0.039	6,339 (1%)	370 (1%)	0.044	460 (0%)	496 (0%)	0.037

The 4 cohorts represent vaccine rollout periods. *Calculated as the days of previous observation in the database before index date. **Assessed anytime before index date. ASMD = Absolute standardized mean difference, GP = General practice, PCR = Polymerase chain reaction, COPD = Chronic obstructive pulmonary disease, GERD = Gastro-Esophageal reflux disease

Table S26: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (any COVID-19 vaccine). Follow-up ends at first vaccine dose after index date.

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 346,674**	**N = 552,602**	**N = 23,982**	**N = 26,736**	**N = 169,100**	**N = 118,507**	**N = 223,962**	**N = 89,941**
	0 to 30 days	**VTE**	93 (2.68)	45 (0.81)	77 (32.11)	6 (2.24)	8 (0.47)	< 5	74 (3.30)	< 5
		DVT	22 (0.63)	10 (0.18)	12 (5.00)	< 5	< 5	< 5	19 (0.85)	< 5
		PE	75 (2.16)	37 (0.67)	67 (27.94)	5 (1.87)	7 (0.41)	< 5	59 (2.63)	< 5
		ATE	22 (0.63)	28 (0.51)	110 (45.87)	6 (2.24)	6 (0.35)	< 5	77 (3.44)	6 (0.67)
		IS	8 (0.23)	< 5	64 (26.69)	< 5	< 5	< 5	45 (2.01)	< 5
		TIA	< 5	6 (0.11)	20 (8.34)	< 5	< 5	< 5	7 (0.31)	< 5
		MI	11 (0.32)	20 (0.36)	35 (14.59)	5 (1.87)	< 5	< 5	27 (1.21)	< 5
		HF	59 (1.70)	73 (1.32)	395 (164.71)	27 (10.10)	10 (0.59)	< 5	302 (13.48)	23 (2.56)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.31)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	31 to 90 days	**VTE**	19 (0.55)	20 (0.36)	37 (15.43)	< 5	< 5	< 5	16 (0.71)	< 5
		DVT	10 (0.29)	7 (0.13)	20 (8.34)	< 5	< 5	< 5	10 (0.45)	< 5
		PE	9 (0.26)	13 (0.24)	21 (8.76)	< 5	< 5	< 5	6 (0.27)	< 5
		ATE	5 (0.14)	16 (0.29)	33 (13.76)	< 5	< 5	< 5	41 (1.83)	5 (0.56)
		IS	< 5	< 5	19 (7.92)	< 5	< 5	< 5	20 (0.89)	< 5
		TIA	< 5	6 (0.11)	8 (3.34)	< 5	< 5	< 5	13 (0.58)	< 5
		MI	< 5	7 (0.13)	12 (5.00)	< 5	< 5	< 5	11 (0.49)	< 5
		HF	30 (0.87)	56 (1.01)	151 (62.96)	14 (5.24)	< 5	< 5	89 (3.97)	8 (0.89)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	**VTE**	10 (0.29)	9 (0.16)	21 (8.76)	5 (1.87)	< 5	< 5	20 (0.89)	< 5
		DVT	5 (0.14)	< 5	14 (5.84)	< 5	< 5	< 5	9 (0.40)	< 5
		PE	5 (0.14)	5 (0.09)	9 (3.75)	< 5	< 5	< 5	11 (0.49)	< 5
		ATE	11 (0.32)	14 (0.25)	31 (12.93)	< 5	< 5	< 5	30 (1.34)	< 5
		IS	< 5	< 5	16 (6.67)	< 5	< 5	< 5	16 (0.71)	< 5
		TIA	5 (0.14)	6 (0.11)	8 (3.34)	< 5	< 5	< 5	8 (0.36)	< 5
		MI	5 (0.14)	< 5	10 (4.17)	< 5	< 5	< 5	7 (0.31)	< 5
		HF	37 (1.07)	51 (0.92)	162 (67.55)	21 (7.85)	< 5	< 5	87 (3.88)	7 (0.78)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	10 (0.29)	8 (0.14)	45 (18.76)	7 (2.62)	< 5	< 5	10 (0.45)	< 5
		DVT	5 (0.14)	< 5	16 (6.67)	< 5	< 5	< 5	6 (0.27)	< 5
		PE	5 (0.14)	< 5	34 (14.18)	< 5	< 5	< 5	< 5	< 5
		ATE	10 (0.29)	19 (0.34)	55 (22.93)	11 (4.11)	< 5	< 5	42 (1.88)	8 (0.89)
		IS	< 5	6 (0.11)	32 (13.34)	10 (3.74)	< 5	< 5	21 (0.94)	5 (0.56)
		TIA	6 (0.17)	< 5	16 (6.67)	< 5	< 5	< 5	10 (0.45)	< 5
		MI	< 5	10 (0.18)	14 (5.84)	< 5	< 5	< 5	11 (0.49)	< 5
		HF	40 (1.15)	53 (0.96)	268 (111.75)	48 (17.95)	< 5	6 (0.51)	86 (3.84)	20 (2.22)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 2			**N = 1,975,726**	**N = 1,563,569**	**N = 34,317**	**N = 4,572**	**N = 583,399**	**N = 486,619**	**N = 433,151**	**N = 819,590**
	0 to 30 days	VTE	241 (1.22)	54 (0.35)	79 (23.02)	< 5	31 (0.53)	7 (0.14)	258 (5.96)	27 (0.33)
		DVT	41 (0.21)	19 (0.12)	12 (3.50)	< 5	8 (0.14)	< 5	63 (1.45)	9 (0.11)
		PE	204 (1.03)	38 (0.24)	69 (20.11)	< 5	24 (0.41)	6 (0.12)	213 (4.92)	20 (0.24)
		ATE	41 (0.21)	25 (0.16)	110 (32.05)	< 5	< 5	< 5	173 (3.99)	29 (0.35)
		IS	7 (0.04)	5 (0.03)	68 (19.82)	< 5	< 5	< 5	96 (2.22)	12 (0.15)
		TIA	8 (0.04)	< 5	18 (5.25)	< 5	< 5	< 5	17 (0.39)	6 (0.07)
		MI	26 (0.13)	17 (0.11)	35 (10.20)	< 5	< 5	< 5	64 (1.48)	11 (0.13)
		HF	45 (0.23)	47 (0.30)	364 (106.07)	< 5	5 (0.09)	< 5	378 (8.73)	76 (0.93)
		HS	6 (0.03)	< 5	< 5	< 5	< 5	< 5	18 (0.42)	< 5
		MP	12 (0.06)	< 5	< 5	< 5	< 5	< 5	14 (0.32)	< 5
	31 to 90 days	VTE	43 (0.22)	13 (0.08)	31 (9.03)	< 5	< 5	< 5	59 (1.36)	9 (0.11)
		DVT	24 (0.12)	6 (0.04)	16 (4.66)	< 5	< 5	< 5	32 (0.74)	5 (0.06)
		PE	23 (0.12)	8 (0.05)	18 (5.25)	< 5	< 5	< 5	33 (0.76)	< 5
		ATE	18 (0.09)	18 (0.12)	32 (9.32)	< 5	< 5	< 5	85 (1.96)	14 (0.17)
		IS	< 5	< 5	20 (5.83)	< 5	< 5	< 5	43 (0.99)	6 (0.07)
		TIA	< 5	7 (0.04)	< 5	< 5	< 5	< 5	23 (0.53)	5 (0.06)
		MI	13 (0.07)	10 (0.06)	13 (3.79)	< 5	< 5	< 5	26 (0.60)	< 5
		HF	27 (0.14)	30 (0.19)	149 (43.42)	< 5	< 5	< 5	138 (3.19)	29 (0.35)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	< 5
		MP	6 (0.03)	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	28 (0.14)	15 (0.10)	26 (7.58)	< 5	6 (0.10)	< 5	58 (1.34)	6 (0.07)
		DVT	13 (0.07)	9 (0.06)	15 (4.37)	< 5	< 5	< 5	31 (0.72)	6 (0.07)
		PE	15 (0.08)	6 (0.04)	12 (3.50)	< 5	< 5	< 5	30 (0.69)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	17 (0.09)	15 (0.10)	32 (9.32)	< 5	< 5	< 5	91 (2.10)	19 (0.23)
		IS	< 5	< 5	15 (4.37)	< 5	< 5	< 5	49 (1.13)	9 (0.11)
		TIA	9 (0.05)	5 (0.03)	11 (3.21)	< 5	< 5	< 5	20 (0.46)	5 (0.06)
		MI	9 (0.05)	10 (0.06)	8 (2.33)	< 5	< 5	< 5	25 (0.58)	6 (0.07)
		HF	22 (0.11)	27 (0.17)	166 (48.37)	< 5	< 5	< 5	110 (2.54)	41 (0.50)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	< 5
	181 to 365 days	VTE	9 (0.05)	12 (0.08)	44 (12.82)	< 5	< 5	< 5	16 (0.37)	23 (0.28)
		DVT	< 5	5 (0.03)	20 (5.83)	< 5	< 5	< 5	9 (0.21)	14 (0.17)
		PE	5 (0.03)	7 (0.04)	27 (7.87)	< 5	< 5	< 5	8 (0.18)	10 (0.12)
		ATE	12 (0.06)	16 (0.10)	53 (15.44)	< 5	< 5	< 5	63 (1.45)	33 (0.40)
		IS	< 5	< 5	31 (9.03)	< 5	< 5	< 5	35 (0.81)	11 (0.13)
		TIA	< 5	< 5	12 (3.50)	< 5	< 5	< 5	16 (0.37)	13 (0.16)
		MI	8 (0.04)	10 (0.06)	15 (4.37)	< 5	< 5	< 5	14 (0.32)	10 (0.12)
		HF	20 (0.10)	31 (0.20)	259 (75.47)	5 (10.94)	< 5	< 5	81 (1.87)	41 (0.50)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	5 (0.06)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,401**	**N = 1,528,031**	**N = 96,423**	**N = 24,050**	**N = 417,996**	**N = 462,832**	**N = 869,497**	**N = 954,232**
	0 to 30 days	VTE	245 (1.62)	25 (0.16)	115 (11.93)	< 5	27 (0.65)	< 5	325 (3.74)	27 (0.28)
		DVT	45 (0.30)	< 5	22 (2.28)	< 5	6 (0.14)	< 5	90 (1.04)	7 (0.07)
		PE	209 (1.38)	22 (0.14)	96 (9.96)	< 5	22 (0.53)	< 5	262 (3.01)	22 (0.23)
		ATE	29 (0.19)	10 (0.07)	119 (12.34)	< 5	< 5	< 5	213 (2.45)	14 (0.15)
		IS	< 5	< 5	70 (7.26)	< 5	< 5	< 5	102 (1.17)	5 (0.05)
		TIA	5 (0.03)	< 5	19 (1.97)	< 5	< 5	< 5	20 (0.23)	< 5
		MI	20 (0.13)	8 (0.05)	40 (4.15)	< 5	< 5	< 5	97 (1.12)	7 (0.07)
		HF	31 (0.21)	18 (0.12)	380 (39.41)	< 5	< 5	< 5	364 (4.19)	23 (0.24)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	20 (0.23)	< 5
		MP	9 (0.06)	< 5	6 (0.62)	< 5	< 5	< 5	19 (0.22)	< 5
	31 to 90 days	VTE	44 (0.29)	< 5	50 (5.19)	< 5	< 5	< 5	85 (0.98)	9 (0.09)
		DVT	20 (0.13)	< 5	28 (2.90)	< 5	< 5	< 5	57 (0.66)	5 (0.05)
		PE	27 (0.18)	< 5	26 (2.70)	< 5	< 5	< 5	38 (0.44)	< 5
		ATE	11 (0.07)	7 (0.05)	48 (4.98)	< 5	< 5	< 5	109 (1.25)	10 (0.10)
		IS	< 5	< 5	24 (2.49)	< 5	< 5	< 5	54 (0.62)	6 (0.06)
		TIA	< 5	< 5	7 (0.73)	< 5	< 5	< 5	27 (0.31)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	< 5	20 (2.07)	< 5	< 5	< 5	36 (0.41)	< 5
		HF	15 (0.10)	8 (0.05)	180 (18.67)	< 5	< 5	< 5	137 (1.58)	13 (0.14)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.08)	< 5
		MP	8 (0.05)	< 5	5 (0.52)	< 5	< 5	< 5	8 (0.09)	< 5
	91 to 180 days	VTE	24 (0.16)	8 (0.05)	43 (4.46)	< 5	< 5	< 5	64 (0.74)	8 (0.08)
		DVT	11 (0.07)	6 (0.04)	26 (2.70)	< 5	< 5	< 5	36 (0.41)	7 (0.07)
		PE	14 (0.09)	< 5	18 (1.87)	< 5	< 5	< 5	32 (0.37)	< 5
		ATE	< 5	7 (0.05)	44 (4.56)	< 5	< 5	< 5	113 (1.30)	20 (0.21)
		IS	< 5	< 5	21 (2.18)	< 5	< 5	< 5	57 (0.66)	12 (0.13)
		TIA	< 5	< 5	13 (1.35)	< 5	< 5	< 5	22 (0.25)	< 5
		MI	< 5	< 5	15 (1.56)	< 5	< 5	< 5	37 (0.43)	6 (0.06)
		HF	11 (0.07)	6 (0.04)	216 (22.40)	< 5	< 5	< 5	120 (1.38)	15 (0.16)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	9 (0.10)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	8 (0.09)	< 5
	181 to 365 days	VTE	< 5	9 (0.06)	72 (7.47)	< 5	< 5	< 5	34 (0.39)	5 (0.05)
		DVT	< 5	< 5	39 (4.04)	< 5	< 5	< 5	20 (0.23)	< 5
		PE	< 5	6 (0.04)	37 (3.84)	< 5	< 5	< 5	16 (0.18)	< 5
		ATE	< 5	< 5	80 (8.30)	< 5	< 5	< 5	51 (0.59)	18 (0.19)
		IS	< 5	< 5	36 (3.73)	< 5	< 5	< 5	24 (0.28)	9 (0.09)
		TIA	< 5	< 5	19 (1.97)	< 5	< 5	< 5	11 (0.13)	< 5
		MI	< 5	< 5	32 (3.32)	< 5	< 5	< 5	17 (0.20)	7 (0.07)
		HF	5 (0.03)	< 5	324 (33.60)	< 5	< 5	< 5	62 (0.71)	13 (0.14)
		HS	< 5	< 5	7 (0.73)	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 4			**N = 2,027,763**	**N = 2,085,598**	**N = 147,545**	**N = 22,245**	**N = 469,876**	**N = 550,437**	**N = 1,061,634**	**N = 880,950**
	0 to 30 days	VTE	334 (1.65)	19 (0.09)	116 (7.86)	< 5	36 (0.77)	7 (0.13)	350 (3.30)	38 (0.43)
		DVT	55 (0.27)	6 (0.03)	22 (1.49)	< 5	6 (0.13)	< 5	106 (1.00)	14 (0.16)
		PE	291 (1.44)	13 (0.06)	97 (6.57)	< 5	31 (0.66)	7 (0.13)	272 (2.56)	25 (0.28)
		ATE	26 (0.13)	5 (0.02)	116 (7.86)	< 5	< 5	< 5	231 (2.18)	35 (0.40)
		IS	< 5	< 5	69 (4.68)	< 5	< 5	< 5	115 (1.08)	17 (0.19)
		TIA	6 (0.03)	< 5	18 (1.22)	< 5	< 5	< 5	26 (0.24)	< 5
		MI	16 (0.08)	< 5	38 (2.58)	< 5	< 5	< 5	96 (0.90)	15 (0.17)
		HF	28 (0.14)	< 5	364 (24.67)	< 5	< 5	< 5	362 (3.41)	28 (0.32)
		HS	< 5	< 5	5 (0.34)	< 5	< 5	< 5	23 (0.22)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	12 (0.06)	< 5	7 (0.47)	< 5	< 5	< 5	26 (0.24)	9 (0.10)
	31 to 90 days	VTE	58 (0.29)	11 (0.05)	54 (3.66)	< 5	5 (0.11)	< 5	91 (0.86)	12 (0.14)
		DVT	21 (0.10)	< 5	32 (2.17)	< 5	< 5	< 5	61 (0.57)	7 (0.08)
		PE	40 (0.20)	7 (0.03)	26 (1.76)	< 5	< 5	< 5	40 (0.38)	6 (0.07)
		ATE	12 (0.06)	< 5	46 (3.12)	< 5	< 5	< 5	118 (1.11)	18 (0.20)
		IS	< 5	< 5	24 (1.63)	< 5	< 5	< 5	52 (0.49)	7 (0.08)
		TIA	< 5	< 5	6 (0.41)	< 5	< 5	< 5	33 (0.31)	7 (0.08)
		MI	9 (0.04)	< 5	19 (1.29)	< 5	< 5	< 5	41 (0.39)	< 5
		HF	14 (0.07)	8 (0.04)	176 (11.93)	< 5	< 5	< 5	142 (1.34)	17 (0.19)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	8 (0.08)	< 5
		MP	7 (0.03)	< 5	5 (0.34)	< 5	< 5	< 5	10 (0.09)	< 5
	91 to 180 days	VTE	26 (0.13)	9 (0.04)	49 (3.32)	< 5	< 5	< 5	71 (0.67)	11 (0.12)
		DVT	10 (0.05)	7 (0.03)	31 (2.10)	< 5	< 5	< 5	42 (0.40)	6 (0.07)
		PE	17 (0.08)	< 5	19 (1.29)	< 5	< 5	< 5	32 (0.30)	6 (0.07)
		ATE	< 5	5 (0.02)	41 (2.78)	< 5	< 5	< 5	128 (1.21)	21 (0.24)
		IS	< 5	< 5	19 (1.29)	< 5	< 5	< 5	65 (0.61)	11 (0.12)
		TIA	< 5	< 5	12 (0.81)	< 5	< 5	< 5	29 (0.27)	< 5
		MI	< 5	< 5	14 (0.95)	< 5	< 5	< 5	37 (0.35)	7 (0.08)
		HF	10 (0.05)	< 5	208 (14.10)	< 5	< 5	< 5	139 (1.31)	19 (0.22)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	14 (0.13)	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	11 (0.10)	< 5
	181 to 365 days	VTE	< 5	< 5	77 (5.22)	< 5	< 5	< 5	46 (0.43)	9 (0.10)
		DVT	< 5	< 5	46 (3.12)	< 5	< 5	< 5	33 (0.31)	5 (0.06)
		PE	< 5	< 5	35 (2.37)	< 5	< 5	< 5	14 (0.13)	< 5
		ATE	< 5	< 5	73 (4.95)	< 5	< 5	< 5	54 (0.51)	22 (0.25)
		IS	< 5	< 5	33 (2.24)	< 5	< 5	< 5	27 (0.25)	15 (0.17)
		TIA	< 5	< 5	19 (1.29)	< 5	< 5	< 5	12 (0.11)	< 5
		MI	< 5	< 5	27 (1.83)	< 5	< 5	< 5	16 (0.15)	7 (0.08)
		HF	< 5	< 5	301 (20.40)	< 5	< 5	< 5	57 (0.54)	11 (0.12)
		HS	< 5	< 5	8 (0.54)	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	7 (0.07)	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S27: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (any COVID-19 vaccine). Only first outcome after COVID-19 captured.

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 346,674**	**N = 552,602**	**N = 23,982**	**N = 26,736**	**N = 169,100**	**N = 118,507**	**N = 223,962**	**N = 89,941**
	0 to 30 days	**VTE**	93 (2.68)	117 (2.12)	77 (32.11)	45 (16.83)	8 (0.47)	8 (0.68)	74 (3.30)	96 (10.67)
		DVT	22 (0.63)	27 (0.49)	12 (5.00)	14 (5.24)	< 5	< 5	19 (0.85)	29 (3.22)
		PE	75 (2.16)	95 (1.72)	67 (27.94)	33 (12.34)	7 (0.41)	7 (0.59)	59 (2.63)	77 (8.56)
		ATE	22 (0.63)	70 (1.27)	110 (45.87)	81 (30.30)	6 (0.35)	7 (0.59)	77 (3.44)	208 (23.13)
		IS	8 (0.23)	5 (0.09)	64 (26.69)	37 (13.84)	< 5	< 5	45 (2.01)	116 (12.90)
		TIA	< 5	18 (0.33)	20 (8.34)	18 (6.73)	< 5	< 5	7 (0.31)	41 (4.56)
		MI	11 (0.32)	46 (0.83)	35 (14.59)	35 (13.09)	< 5	5 (0.42)	27 (1.21)	63 (7.00)
		HF	59 (1.70)	198 (3.58)	395 (164.71)	299 (111.83)	10 (0.59)	9 (0.76)	302 (13.48)	640 (71.16)
		HS	< 5	7 (0.13)	< 5	< 5	< 5	< 5	7 (0.31)	14 (1.56)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	31 to 90 days	**VTE**	19 (0.55)	39 (0.71)	35 (14.59)	29 (10.85)	< 5	< 5	15 (0.67)	45 (5.00)
		DVT	10 (0.29)	15 (0.27)	19 (7.92)	15 (5.61)	< 5	< 5	9 (0.40)	29 (3.22)
		PE	9 (0.26)	25 (0.45)	20 (8.34)	14 (5.24)	< 5	< 5	6 (0.27)	20 (2.22)
		ATE	5 (0.14)	43 (0.78)	29 (12.09)	44 (16.46)	< 5	< 5	39 (1.74)	127 (14.12)
		IS	< 5	< 5	16 (6.67)	19 (7.11)	< 5	< 5	20 (0.89)	70 (7.78)
		TIA	< 5	19 (0.34)	8 (3.34)	17 (6.36)	< 5	< 5	13 (0.58)	32 (3.56)
		MI	< 5	20 (0.36)	10 (4.17)	11 (4.11)	< 5	< 5	9 (0.40)	29 (3.22)
		HF	30 (0.87)	109 (1.97)	134 (55.88)	162 (60.59)	< 5	8 (0.68)	85 (3.80)	290 (32.24)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	12 (1.33)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	**VTE**	10 (0.29)	18 (0.33)	16 (6.67)	29 (10.85)	< 5	< 5	18 (0.80)	35 (3.89)
		DVT	5 (0.14)	8 (0.14)	9 (3.75)	16 (5.98)	< 5	< 5	7 (0.31)	19 (2.11)
		PE	5 (0.14)	10 (0.18)	9 (3.75)	15 (5.61)	< 5	< 5	11 (0.49)	18 (2.00)
		ATE	11 (0.32)	28 (0.51)	28 (11.68)	45 (16.83)	< 5	6 (0.51)	25 (1.12)	104 (11.56)
		IS	< 5	8 (0.14)	13 (5.42)	19 (7.11)	< 5	< 5	13 (0.58)	57 (6.34)
		TIA	5 (0.14)	12 (0.22)	8 (3.34)	15 (5.61)	< 5	< 5	7 (0.31)	34 (3.78)
		MI	5 (0.14)	8 (0.14)	9 (3.75)	16 (5.98)	< 5	5 (0.42)	6 (0.27)	23 (2.56)
		HF	35 (1.01)	89 (1.61)	143 (59.63)	203 (75.93)	< 5	5 (0.42)	81 (3.62)	228 (25.35)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	9 (0.26)	11 (0.20)	29 (12.09)	27 (10.10)	< 5	< 5	5 (0.22)	11 (1.22)
		DVT	< 5	6 (0.11)	10 (4.17)	17 (6.36)	< 5	< 5	< 5	7 (0.78)
		PE	5 (0.14)	5 (0.09)	24 (10.01)	11 (4.11)	< 5	< 5	< 5	< 5
		ATE	10 (0.29)	23 (0.42)	45 (18.76)	74 (27.68)	< 5	< 5	35 (1.56)	39 (4.34)
		IS	< 5	6 (0.11)	26 (10.84)	34 (12.72)	< 5	< 5	18 (0.80)	24 (2.67)
		TIA	6 (0.17)	7 (0.13)	15 (6.25)	29 (10.85)	< 5	< 5	9 (0.40)	9 (1.00)
		MI	< 5	11 (0.20)	10 (4.17)	17 (6.36)	< 5	< 5	8 (0.36)	7 (0.78)
		HF	36 (1.04)	53 (0.96)	213 (88.82)	292 (109.22)	< 5	6 (0.51)	75 (3.35)	117 (13.01)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.78)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 2			**N = 1,975,726**	**N = 1,563,569**	**N = 34,317**	**N = 4,572**	**N = 583,399**	**N = 486,619**	**N = 433,151**	**N = 819,590**
	0 to 30 days	VTE	241 (1.22)	220 (1.41)	79 (23.02)	7 (15.31)	31 (0.53)	24 (0.49)	258 (5.96)	400 (4.88)
		DVT	41 (0.21)	65 (0.42)	12 (3.50)	< 5	8 (0.14)	5 (0.10)	63 (1.45)	165 (2.01)
		PE	204 (1.03)	165 (1.06)	69 (20.11)	5 (10.94)	24 (0.41)	19 (0.39)	213 (4.92)	269 (3.28)
		ATE	41 (0.21)	104 (0.67)	110 (32.05)	5 (10.94)	< 5	6 (0.12)	173 (3.99)	669 (8.16)
		IS	7 (0.04)	14 (0.09)	68 (19.82)	< 5	< 5	< 5	96 (2.22)	346 (4.22)
		TIA	8 (0.04)	24 (0.15)	18 (5.25)	< 5	< 5	< 5	17 (0.39)	122 (1.49)
		MI	26 (0.13)	68 (0.43)	35 (10.20)	< 5	< 5	6 (0.12)	64 (1.48)	239 (2.92)
		HF	45 (0.23)	146 (0.93)	364 (106.07)	23 (50.31)	5 (0.09)	13 (0.27)	378 (8.73)	1,331 (16.24)
		HS	6 (0.03)	5 (0.03)	< 5	< 5	< 5	< 5	18 (0.42)	77 (0.94)
		MP	12 (0.06)	7 (0.04)	< 5	< 5	< 5	< 5	14 (0.32)	37 (0.45)
	31 to 90 days	VTE	41 (0.21)	75 (0.48)	28 (8.16)	5 (10.94)	< 5	9 (0.18)	56 (1.29)	187 (2.28)
		DVT	23 (0.12)	32 (0.20)	15 (4.37)	5 (10.94)	< 5	5 (0.10)	30 (0.69)	119 (1.45)
		PE	22 (0.11)	45 (0.29)	16 (4.66)	< 5	< 5	< 5	31 (0.72)	87 (1.06)
		ATE	18 (0.09)	92 (0.59)	29 (8.45)	< 5	< 5	9 (0.18)	84 (1.94)	437 (5.33)
		IS	< 5	11 (0.07)	18 (5.25)	< 5	< 5	< 5	43 (0.99)	231 (2.82)
		TIA	< 5	33 (0.21)	< 5	< 5	< 5	6 (0.12)	23 (0.53)	115 (1.40)
		MI	13 (0.07)	50 (0.32)	11 (3.21)	< 5	< 5	< 5	25 (0.58)	113 (1.38)
		HF	27 (0.14)	99 (0.63)	137 (39.92)	19 (41.56)	< 5	7 (0.14)	132 (3.05)	622 (7.59)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	27 (0.33)
		MP	6 (0.03)	< 5	< 5	< 5	< 5	< 5	< 5	8 (0.10)
	91 to 180 days	VTE	25 (0.13)	37 (0.24)	21 (6.12)	6 (13.12)	6 (0.10)	< 5	53 (1.22)	109 (1.33)
		DVT	12 (0.06)	21 (0.13)	10 (2.91)	< 5	< 5	< 5	29 (0.67)	71 (0.87)
		PE	13 (0.07)	19 (0.12)	12 (3.50)	< 5	< 5	< 5	27 (0.62)	46 (0.56)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	15 (0.08)	43 (0.28)	31 (9.03)	< 5	< 5	< 5	81 (1.87)	394 (4.81)
		IS	< 5	< 5	14 (4.08)	< 5	< 5	< 5	43 (0.99)	202 (2.46)
		TIA	8 (0.04)	18 (0.12)	11 (3.21)	< 5	< 5	< 5	19 (0.44)	109 (1.33)
		MI	8 (0.04)	25 (0.16)	8 (2.33)	< 5	< 5	< 5	22 (0.51)	104 (1.27)
		HF	22 (0.11)	65 (0.42)	149 (43.42)	20 (43.74)	< 5	< 5	100 (2.31)	534 (6.52)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	35 (0.43)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.16)	9 (0.11)
	181 to 365 days	VTE	8 (0.04)	12 (0.08)	30 (8.74)	5 (10.94)	< 5	< 5	14 (0.32)	52 (0.63)
		DVT	< 5	5 (0.03)	15 (4.37)	< 5	< 5	< 5	8 (0.18)	36 (0.44)
		PE	< 5	8 (0.05)	18 (5.25)	< 5	< 5	< 5	7 (0.16)	19 (0.23)
		ATE	10 (0.05)	17 (0.11)	44 (12.82)	< 5	< 5	< 5	53 (1.22)	151 (1.84)
		IS	< 5	< 5	25 (7.29)	< 5	< 5	< 5	30 (0.69)	71 (0.87)
		TIA	< 5	5 (0.03)	12 (3.50)	< 5	< 5	< 5	14 (0.32)	46 (0.56)
		MI	6 (0.03)	10 (0.06)	11 (3.21)	< 5	< 5	< 5	11 (0.25)	45 (0.55)
		HF	19 (0.10)	34 (0.22)	210 (61.19)	29 (63.43)	< 5	< 5	72 (1.66)	209 (2.55)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	10 (0.12)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,401**	**N = 1,528,031**	**N = 96,423**	**N = 24,050**	**N = 417,996**	**N = 462,832**	**N = 869,497**	**N = 954,232**
	0 to 30 days	VTE	245 (1.62)	142 (0.93)	115 (11.93)	9 (3.74)	27 (0.65)	17 (0.37)	325 (3.74)	180 (1.89)
		DVT	45 (0.30)	41 (0.27)	22 (2.28)	7 (2.91)	6 (0.14)	< 5	90 (1.04)	78 (0.82)
		PE	209 (1.38)	105 (0.69)	96 (9.96)	< 5	22 (0.53)	13 (0.28)	262 (3.01)	118 (1.24)
		ATE	29 (0.19)	49 (0.32)	119 (12.34)	12 (4.99)	< 5	12 (0.26)	213 (2.45)	275 (2.88)
		IS	< 5	5 (0.03)	70 (7.26)	9 (3.74)	< 5	< 5	102 (1.17)	130 (1.36)
		TIA	5 (0.03)	17 (0.11)	19 (1.97)	< 5	< 5	< 5	20 (0.23)	53 (0.56)
		MI	20 (0.13)	27 (0.18)	40 (4.15)	< 5	< 5	9 (0.19)	97 (1.12)	113 (1.18)
		HF	31 (0.21)	38 (0.25)	380 (39.41)	23 (9.56)	< 5	< 5	364 (4.19)	256 (2.68)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	20 (0.23)	35 (0.37)
		MP	9 (0.06)	6 (0.04)	6 (0.62)	< 5	< 5	< 5	19 (0.22)	20 (0.21)
	31 to 90 days	VTE	41 (0.27)	46 (0.30)	45 (4.67)	10 (4.16)	< 5	6 (0.13)	81 (0.93)	90 (0.94)
		DVT	19 (0.13)	27 (0.18)	25 (2.59)	7 (2.91)	< 5	< 5	54 (0.62)	60 (0.63)
		PE	25 (0.17)	19 (0.12)	23 (2.39)	< 5	< 5	< 5	36 (0.41)	37 (0.39)
		ATE	11 (0.07)	33 (0.22)	45 (4.67)	8 (3.33)	< 5	8 (0.17)	108 (1.24)	207 (2.17)
		IS	< 5	< 5	22 (2.28)	< 5	< 5	< 5	54 (0.62)	87 (0.91)
		TIA	< 5	14 (0.09)	7 (0.73)	< 5	< 5	< 5	27 (0.31)	35 (0.37)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	16 (0.10)	18 (1.87)	< 5	< 5	5 (0.11)	35 (0.40)	93 (0.97)
		HF	15 (0.10)	26 (0.17)	166 (17.22)	25 (10.40)	< 5	< 5	130 (1.50)	154 (1.61)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.08)	19 (0.20)
		MP	8 (0.05)	8 (0.05)	< 5	< 5	< 5	< 5	8 (0.09)	11 (0.12)
	91 to 180 days	VTE	23 (0.15)	25 (0.16)	34 (3.53)	11 (4.57)	< 5	< 5	57 (0.66)	92 (0.96)
		DVT	10 (0.07)	15 (0.10)	20 (2.07)	7 (2.91)	< 5	< 5	33 (0.38)	61 (0.64)
		PE	14 (0.09)	13 (0.09)	15 (1.56)	< 5	< 5	< 5	28 (0.32)	34 (0.36)
		ATE	< 5	28 (0.18)	40 (4.15)	8 (3.33)	< 5	< 5	102 (1.17)	199 (2.09)
		IS	< 5	< 5	20 (2.07)	< 5	< 5	< 5	49 (0.56)	92 (0.96)
		TIA	< 5	9 (0.06)	13 (1.35)	< 5	< 5	< 5	20 (0.23)	47 (0.49)
		MI	< 5	18 (0.12)	13 (1.35)	< 5	< 5	< 5	35 (0.40)	69 (0.72)
		HF	11 (0.07)	12 (0.08)	194 (20.12)	27 (11.23)	< 5	< 5	110 (1.27)	133 (1.39)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	9 (0.10)	13 (0.14)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	8 (0.09)	14 (0.15)
	181 to 365 days	VTE	< 5	9 (0.06)	50 (5.19)	16 (6.65)	< 5	< 5	31 (0.36)	19 (0.20)
		DVT	< 5	< 5	29 (3.01)	13 (5.41)	< 5	< 5	18 (0.21)	14 (0.15)
		PE	< 5	6 (0.04)	24 (2.49)	< 5	< 5	< 5	15 (0.17)	6 (0.06)
		ATE	< 5	< 5	66 (6.84)	7 (2.91)	< 5	< 5	42 (0.48)	58 (0.61)
		IS	< 5	< 5	30 (3.11)	< 5	< 5	< 5	19 (0.22)	22 (0.23)
		TIA	< 5	< 5	17 (1.76)	< 5	< 5	< 5	10 (0.12)	7 (0.07)
		MI	< 5	< 5	24 (2.49)	< 5	< 5	< 5	14 (0.16)	32 (0.34)
		HF	5 (0.03)	< 5	262 (27.17)	31 (12.89)	< 5	< 5	54 (0.62)	36 (0.38)
		HS	< 5	< 5	7 (0.73)	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 4			**N = 2,027,763**	**N = 2,085,598**	**N = 147,545**	**N = 22,245**	**N = 469,876**	**N = 550,437**	**N = 1,061,634**	**N = 880,950**
	0 to 30 days	VTE	334 (1.65)	50 (0.24)	116 (7.86)	< 5	36 (0.77)	11 (0.20)	350 (3.30)	98 (1.11)
		DVT	55 (0.27)	17 (0.08)	22 (1.49)	< 5	6 (0.13)	< 5	106 (1.00)	48 (0.54)
		PE	291 (1.44)	34 (0.16)	97 (6.57)	< 5	31 (0.66)	8 (0.15)	272 (2.56)	55 (0.62)
		ATE	26 (0.13)	8 (0.04)	116 (7.86)	10 (4.50)	< 5	< 5	231 (2.18)	95 (1.08)
		IS	< 5	< 5	69 (4.68)	< 5	< 5	< 5	115 (1.08)	47 (0.53)
		TIA	6 (0.03)	< 5	18 (1.22)	< 5	< 5	< 5	26 (0.24)	12 (0.14)
		MI	16 (0.08)	< 5	38 (2.58)	< 5	< 5	< 5	96 (0.90)	39 (0.44)
		HF	28 (0.14)	< 5	364 (24.67)	17 (7.64)	< 5	< 5	362 (3.41)	75 (0.85)
		HS	< 5	< 5	5 (0.34)	< 5	< 5	< 5	23 (0.22)	13 (0.15)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	12 (0.06)	8 (0.04)	7 (0.47)	< 5	< 5	< 5	26 (0.24)	17 (0.19)
	31 to 90 days	VTE	55 (0.27)	22 (0.11)	49 (3.32)	< 5	5 (0.11)	< 5	86 (0.81)	48 (0.54)
		DVT	20 (0.10)	12 (0.06)	29 (1.97)	< 5	< 5	< 5	57 (0.54)	32 (0.36)
		PE	38 (0.19)	10 (0.05)	23 (1.56)	< 5	< 5	< 5	38 (0.36)	17 (0.19)
		ATE	12 (0.06)	9 (0.04)	43 (2.91)	5 (2.25)	< 5	< 5	117 (1.10)	75 (0.85)
		IS	< 5	< 5	22 (1.49)	< 5	< 5	< 5	52 (0.49)	30 (0.34)
		TIA	< 5	< 5	6 (0.41)	< 5	< 5	< 5	33 (0.31)	23 (0.26)
		MI	9 (0.04)	7 (0.03)	17 (1.15)	< 5	< 5	< 5	40 (0.38)	25 (0.28)
		HF	14 (0.07)	9 (0.04)	162 (10.98)	13 (5.84)	< 5	< 5	135 (1.27)	47 (0.53)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	8 (0.08)	5 (0.06)
		MP	7 (0.03)	< 5	< 5	< 5	< 5	< 5	10 (0.09)	11 (0.12)
	91 to 180 days	VTE	25 (0.12)	9 (0.04)	40 (2.71)	< 5	< 5	< 5	65 (0.61)	52 (0.59)
		DVT	9 (0.04)	7 (0.03)	25 (1.69)	< 5	< 5	< 5	39 (0.37)	33 (0.37)
		PE	17 (0.08)	< 5	16 (1.08)	< 5	< 5	< 5	29 (0.27)	22 (0.25)
		ATE	< 5	6 (0.03)	38 (2.58)	6 (2.70)	< 5	< 5	116 (1.09)	86 (0.98)
		IS	< 5	< 5	18 (1.22)	< 5	< 5	< 5	57 (0.54)	42 (0.48)
		TIA	< 5	< 5	12 (0.81)	< 5	< 5	< 5	26 (0.24)	17 (0.19)
		MI	< 5	< 5	12 (0.81)	< 5	< 5	< 5	35 (0.33)	27 (0.31)
		HF	10 (0.05)	< 5	187 (12.67)	18 (8.09)	< 5	< 5	127 (1.20)	54 (0.61)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	13 (0.12)	6 (0.07)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	11 (0.10)	6 (0.07)
	181 to 365 days	VTE	< 5	< 5	53 (3.59)	< 5	< 5	< 5	42 (0.40)	8 (0.09)
		DVT	< 5	< 5	33 (2.24)	< 5	< 5	< 5	30 (0.28)	6 (0.07)
		PE	< 5	< 5	23 (1.56)	< 5	< 5	< 5	13 (0.12)	< 5
		ATE	< 5	< 5	64 (4.34)	9 (4.05)	< 5	< 5	42 (0.40)	16 (0.18)
		IS	< 5	< 5	29 (1.97)	5 (2.25)	< 5	< 5	20 (0.19)	11 (0.12)
		TIA	< 5	< 5	19 (1.29)	< 5	< 5	< 5	10 (0.09)	< 5
		MI	< 5	< 5	21 (1.42)	< 5	< 5	< 5	13 (0.12)	5 (0.06)
		HF	< 5	< 5	243 (16.47)	12 (5.39)	< 5	< 5	47 (0.44)	11 (0.12)
		HS	< 5	< 5	8 (0.54)	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.07)	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S28: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (ChAdOx1 vaccine).

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 344,687**	**N = 219,804**	**N = 168,972**	**N = 82,406**		
	0 to 30 days	**VTE**	77 (2.23)	41 (1.87)	7 (0.41)	< 5		
		DVT	16 (0.46)	12 (0.55)	< 5	< 5		
		PE	64 (1.86)	31 (1.41)	6 (0.36)	< 5		
		ATE	18 (0.52)	25 (1.14)	7 (0.41)	6 (0.73)		
		IS	6 (0.17)	< 5	< 5	< 5		
		TIA	< 5	7 (0.32)	< 5	< 5		
		MI	9 (0.26)	17 (0.77)	5 (0.30)	< 5		
		HF	42 (1.22)	72 (3.28)	9 (0.53)	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	31 to 90 days	**VTE**	12 (0.35)	14 (0.64)	< 5	< 5		
		DVT	5 (0.15)	8 (0.36)	< 5	< 5		
		PE	7 (0.20)	7 (0.32)	< 5	< 5		
		ATE	6 (0.17)	20 (0.91)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	9 (0.41)	< 5	< 5		
		MI	5 (0.15)	9 (0.41)	< 5	< 5		
		HF	26 (0.75)	42 (1.91)	< 5	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	91 to 180 days	**VTE**	6 (0.17)	15 (0.68)	< 5	< 5		
		DVT	< 5	5 (0.23)	< 5	< 5		
		PE	< 5	10 (0.45)	< 5	< 5		
		ATE	10 (0.29)	10 (0.45)	< 5	6 (0.73)		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.15)	6 (0.27)	< 5	< 5		
		MI	< 5	< 5	< 5	5 (0.61)		
		HF	31 (0.90)	38 (1.73)	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	5 (0.15)	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	9 (0.26)	9 (0.41)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	35 (1.02)	20 (0.91)	< 5	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
Cohort 2			N = 1,975,770	N = 969,262	N = 582,791	N = 302,999	N = 433,636	N = 323,204
	0 to 30 days	VTE	243 (1.23)	158 (1.63)	33 (0.57)	19 (0.63)	262 (6.04)	90 (2.78)
		DVT	42 (0.21)	47 (0.48)	8 (0.14)	< 5	65 (1.50)	45 (1.39)
		PE	206 (1.04)	120 (1.24)	26 (0.45)	15 (0.50)	218 (5.03)	55 (1.70)
		ATE	38 (0.19)	75 (0.77)	< 5	< 5	177 (4.08)	131 (4.05)
		IS	6 (0.03)	12 (0.12)	< 5	< 5	97 (2.24)	52 (1.61)
		TIA	7 (0.04)	13 (0.13)	< 5	< 5	17 (0.39)	23 (0.71)
		MI	25 (0.13)	52 (0.54)	< 5	< 5	67 (1.55)	62 (1.92)
		HF	46 (0.23)	103 (1.06)	5 (0.09)	11 (0.36)	379 (8.74)	123 (3.81)
		HS	5 (0.03)	< 5	< 5	< 5	21 (0.48)	7 (0.22)
		MP	11 (0.06)	6 (0.06)	< 5	< 5	15 (0.35)	10 (0.31)
	31 to 90 days	VTE	49 (0.25)	55 (0.57)	< 5	9 (0.30)	60 (1.38)	36 (1.11)
		DVT	25 (0.13)	25 (0.26)	< 5	5 (0.17)	32 (0.74)	25 (0.77)
		PE	27 (0.14)	32 (0.33)	< 5	< 5	34 (0.78)	18 (0.56)
		ATE	16 (0.08)	57 (0.59)	< 5	5 (0.17)	86 (1.98)	107 (3.31)
		IS	< 5	6 (0.06)	< 5	< 5	42 (0.97)	58 (1.79)
		TIA	< 5	25 (0.26)	< 5	< 5	23 (0.53)	24 (0.74)
		MI	11 (0.06)	28 (0.29)	< 5	< 5	28 (0.65)	27 (0.84)
		HF	24 (0.12)	68 (0.70)	< 5	7 (0.23)	140 (3.23)	62 (1.92)
		HS	< 5	< 5	< 5	< 5	7 (0.16)	7 (0.22)
		MP	6 (0.03)	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	25 (0.13)	30 (0.31)	< 5	< 5	58 (1.34)	23 (0.71)
		DVT	11 (0.06)	16 (0.17)	< 5	< 5	31 (0.71)	16 (0.50)
		PE	14 (0.07)	16 (0.17)	< 5	< 5	29 (0.67)	8 (0.25)

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	12 (0.06)	28 (0.29)	< 5	< 5	94 (2.17)	106 (3.28)
		IS	< 5	< 5	< 5	< 5	51 (1.18)	40 (1.24)
		TIA	7 (0.04)	13 (0.13)	< 5	< 5	21 (0.48)	32 (0.99)
		MI	6 (0.03)	15 (0.15)	< 5	< 5	25 (0.58)	37 (1.14)
		HF	16 (0.08)	44 (0.45)	< 5	< 5	115 (2.65)	60 (1.86)
		HS	< 5	< 5	< 5	< 5	8 (0.18)	< 5
		MP	< 5	< 5	< 5	< 5	8 (0.18)	< 5
	181 to 365 days	VTE	14 (0.07)	5 (0.05)	< 5	< 5	18 (0.42)	16 (0.50)
		DVT	< 5	< 5	< 5	< 5	10 (0.23)	13 (0.40)
		PE	10 (0.05)	< 5	< 5	< 5	9 (0.21)	< 5
		ATE	11 (0.06)	11 (0.11)	< 5	< 5	71 (1.64)	46 (1.42)
		IS	< 5	< 5	< 5	< 5	38 (0.88)	21 (0.65)
		TIA	< 5	< 5	< 5	< 5	20 (0.46)	13 (0.40)
		MI	8 (0.04)	7 (0.07)	< 5	< 5	15 (0.35)	16 (0.50)
		HF	18 (0.09)	23 (0.24)	< 5	< 5	81 (1.87)	26 (0.80)
		HS	< 5	< 5	< 5	< 5	5 (0.12)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,493**	**N = 1,473,602**	**N = 418,184**	**N = 423,876**	**N = 873,400**	**N = 84,204**
	0 to 30 days	VTE	244 (1.62)	139 (0.94)	28 (0.67)	16 (0.38)	332 (3.80)	27 (3.21)
		DVT	44 (0.29)	41 (0.28)	6 (0.14)	< 5	95 (1.09)	9 (1.07)
		PE	209 (1.38)	101 (0.69)	23 (0.55)	12 (0.28)	266 (3.05)	22 (2.61)
		ATE	28 (0.19)	47 (0.32)	< 5	12 (0.28)	216 (2.47)	32 (3.80)
		IS	< 5	5 (0.03)	< 5	< 5	103 (1.18)	13 (1.54)
		TIA	5 (0.03)	16 (0.11)	< 5	< 5	22 (0.25)	5 (0.59)
		MI	19 (0.13)	26 (0.18)	< 5	9 (0.21)	97 (1.11)	16 (1.90)
		HF	30 (0.20)	28 (0.19)	< 5	< 5	372 (4.26)	51 (6.06)
		HS	< 5	< 5	< 5	< 5	20 (0.23)	< 5
		MP	9 (0.06)	6 (0.04)	< 5	< 5	20 (0.23)	6 (0.71)
	31 to 90 days	VTE	46 (0.30)	44 (0.30)	< 5	7 (0.17)	85 (0.97)	9 (1.07)
		DVT	21 (0.14)	26 (0.18)	< 5	< 5	57 (0.65)	< 5
		PE	28 (0.19)	18 (0.12)	< 5	< 5	38 (0.44)	6 (0.71)
		ATE	11 (0.07)	31 (0.21)	< 5	7 (0.17)	109 (1.25)	25 (2.97)
		IS	< 5	< 5	< 5	< 5	52 (0.60)	11 (1.31)
		TIA	< 5	13 (0.09)	< 5	< 5	27 (0.31)	< 5

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	16 (0.11)	< 5	< 5	38 (0.44)	10 (1.19)
		HF	14 (0.09)	23 (0.16)	< 5	< 5	142 (1.63)	25 (2.97)
		HS	< 5	< 5	< 5	< 5	7 (0.08)	< 5
		MP	8 (0.05)	7 (0.05)	< 5	< 5	9 (0.10)	< 5
	91 to 180 days	VTE	24 (0.16)	26 (0.18)	< 5	< 5	65 (0.74)	14 (1.66)
		DVT	11 (0.07)	15 (0.10)	< 5	< 5	36 (0.41)	7 (0.83)
		PE	14 (0.09)	14 (0.10)	< 5	< 5	33 (0.38)	8 (0.95)
		ATE	< 5	26 (0.18)	< 5	< 5	115 (1.32)	30 (3.56)
		IS	< 5	< 5	< 5	< 5	58 (0.66)	14 (1.66)
		TIA	< 5	8 (0.05)	< 5	< 5	22 (0.25)	10 (1.19)
		MI	< 5	17 (0.12)	< 5	< 5	38 (0.44)	8 (0.95)
		HF	10 (0.07)	12 (0.08)	< 5	< 5	122 (1.40)	12 (1.43)
		HS	< 5	< 5	< 5	< 5	9 (0.10)	< 5
		MP	< 5	< 5	< 5	< 5	8 (0.09)	< 5
	181 to 365 days	VTE	< 5	11 (0.07)	< 5	< 5	35 (0.40)	7 (0.83)
		DVT	< 5	5 (0.03)	< 5	< 5	21 (0.24)	< 5
		PE	< 5	7 (0.05)	< 5	< 5	16 (0.18)	5 (0.59)
		ATE	< 5	< 5	< 5	< 5	57 (0.65)	15 (1.78)
		IS	< 5	< 5	< 5	< 5	26 (0.30)	6 (0.71)
		TIA	< 5	< 5	< 5	< 5	12 (0.14)	< 5
		MI	< 5	< 5	< 5	< 5	20 (0.23)	8 (0.95)
		HF	< 5	< 5	< 5	< 5	64 (0.73)	6 (0.71)
		HS	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 4			N = 2,066,318	N = 542,670	N = 485,154	N = 147,744		
	0 to 30 days	VTE	346 (1.67)	27 (0.50)	38 (0.78)	8 (0.54)		
		DVT	56 (0.27)	7 (0.13)	6 (0.12)	< 5		
		PE	302 (1.46)	21 (0.39)	33 (0.68)	6 (0.41)		
		ATE	26 (0.13)	5 (0.09)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.02)	< 5	< 5	< 5		
		MI	17 (0.08)	< 5	< 5	< 5		
		HF	28 (0.14)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	14 (0.07)	< 5	< 5	< 5		
	31 to 90 days	VTE	60 (0.29)	14 (0.26)	< 5	< 5		
		DVT	23 (0.11)	8 (0.15)	< 5	< 5		
		PE	40 (0.19)	6 (0.11)	< 5	< 5		
		ATE	13 (0.06)	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	9 (0.04)	< 5	< 5	< 5		
		HF	14 (0.07)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	7 (0.03)	< 5	< 5	< 5		
	91 to 180 days	VTE	29 (0.14)	< 5	< 5	< 5		
		DVT	10 (0.05)	< 5	< 5	< 5		
		PE	20 (0.10)	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	10 (0.05)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	181 to 365 days	VTE	< 5	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	< 5	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S29: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (ChAdOx1 vaccine). Follow-up ends at first vaccine dose after index date.

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			N = 344,687	N = 219,804	N = 168,972	N = 82,406		
	0 to 30 days	VTE	77 (2.23)	14 (0.64)	7 (0.41)	< 5		
		DVT	16 (0.46)	< 5	< 5	< 5		
		PE	64 (1.86)	13 (0.59)	6 (0.36)	< 5		
		ATE	18 (0.52)	10 (0.45)	7 (0.41)	< 5		
		IS	6 (0.17)	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	9 (0.26)	6 (0.27)	5 (0.30)	< 5		
		HF	42 (1.22)	28 (1.27)	9 (0.53)	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	31 to 90 days	VTE	12 (0.35)	5 (0.23)	< 5	< 5		
		DVT	5 (0.15)	< 5	< 5	< 5		
		PE	7 (0.20)	< 5	< 5	< 5		
		ATE	6 (0.17)	8 (0.36)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	5 (0.15)	< 5	< 5	< 5		
		HF	26 (0.75)	20 (0.91)	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	91 to 180 days	VTE	6 (0.17)	5 (0.23)	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	10 (0.29)	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.15)	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	31 (0.90)	19 (0.86)	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	5 (0.15)	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	9 (0.26)	8 (0.36)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	35 (1.02)	18 (0.82)	< 5	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
Cohort 2			N = 1,975,770	N = 969,262	N = 582,791	N = 302,999	N = 433,636	N = 323,204
	0 to 30 days	VTE	243 (1.23)	40 (0.41)	33 (0.57)	< 5	262 (6.04)	9 (0.28)
		DVT	42 (0.21)	13 (0.13)	8 (0.14)	< 5	65 (1.50)	5 (0.15)
		PE	206 (1.04)	30 (0.31)	26 (0.45)	< 5	218 (5.03)	6 (0.19)
		ATE	38 (0.19)	11 (0.11)	< 5	< 5	177 (4.08)	6 (0.19)
		IS	6 (0.03)	< 5	< 5	< 5	97 (2.24)	< 5
		TIA	7 (0.04)	< 5	< 5	< 5	17 (0.39)	< 5
		MI	25 (0.13)	9 (0.09)	< 5	< 5	67 (1.55)	< 5
		HF	46 (0.23)	32 (0.33)	5 (0.09)	< 5	379 (8.74)	12 (0.37)
		HS	5 (0.03)	< 5	< 5	< 5	21 (0.48)	< 5
		MP	11 (0.06)	< 5	< 5	< 5	15 (0.35)	< 5
	31 to 90 days	VTE	49 (0.25)	8 (0.08)	< 5	< 5	60 (1.38)	< 5
		DVT	25 (0.13)	5 (0.05)	< 5	< 5	32 (0.74)	< 5
		PE	27 (0.14)	< 5	< 5	< 5	34 (0.78)	< 5
		ATE	16 (0.08)	9 (0.09)	< 5	< 5	86 (1.98)	6 (0.19)
		IS	< 5	< 5	< 5	< 5	42 (0.97)	< 5
		TIA	< 5	< 5	< 5	< 5	23 (0.53)	< 5
		MI	11 (0.06)	6 (0.06)	< 5	< 5	28 (0.65)	< 5
		HF	24 (0.12)	13 (0.13)	< 5	< 5	140 (3.23)	7 (0.22)
		HS	< 5	< 5	< 5	< 5	7 (0.16)	< 5
		MP	6 (0.03)	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	25 (0.13)	12 (0.12)	< 5	< 5	58 (1.34)	< 5
		DVT	11 (0.06)	7 (0.07)	< 5	< 5	31 (0.71)	< 5
		PE	14 (0.07)	5 (0.05)	< 5	< 5	29 (0.67)	< 5

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	12 (0.06)	10 (0.10)	< 5	< 5	94 (2.17)	6 (0.19)
		IS	< 5	< 5	< 5	< 5	51 (1.18)	< 5
		TIA	7 (0.04)	< 5	< 5	< 5	21 (0.48)	< 5
		MI	6 (0.03)	6 (0.06)	< 5	< 5	25 (0.58)	< 5
		HF	16 (0.08)	18 (0.19)	< 5	< 5	115 (2.65)	< 5
		HS	< 5	< 5	< 5	< 5	8 (0.18)	< 5
		MP	< 5	< 5	< 5	< 5	8 (0.18)	< 5
	181 to 365 days	VTE	14 (0.07)	5 (0.05)	< 5	< 5	18 (0.42)	9 (0.28)
		DVT	< 5	< 5	< 5	< 5	10 (0.23)	7 (0.22)
		PE	10 (0.05)	< 5	< 5	< 5	9 (0.21)	< 5
		ATE	11 (0.06)	10 (0.10)	< 5	< 5	71 (1.64)	10 (0.31)
		IS	< 5	< 5	< 5	< 5	38 (0.88)	< 5
		TIA	< 5	< 5	< 5	< 5	20 (0.46)	< 5
		MI	8 (0.04)	6 (0.06)	< 5	< 5	15 (0.35)	5 (0.15)
		HF	18 (0.09)	20 (0.21)	< 5	< 5	81 (1.87)	9 (0.28)
		HS	< 5	< 5	< 5	< 5	5 (0.12)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,493**	**N = 1,473,602**	**N = 418,184**	**N = 423,876**	**N = 873,400**	**N = 84,204**
	0 to 30 days	VTE	244 (1.62)	23 (0.16)	28 (0.67)	< 5	332 (3.80)	5 (0.59)
		DVT	44 (0.29)	< 5	6 (0.14)	< 5	95 (1.09)	< 5
		PE	209 (1.38)	19 (0.13)	23 (0.55)	< 5	266 (3.05)	< 5
		ATE	28 (0.19)	8 (0.05)	< 5	< 5	216 (2.47)	< 5
		IS	< 5	< 5	< 5	< 5	103 (1.18)	< 5
		TIA	5 (0.03)	< 5	< 5	< 5	22 (0.25)	< 5
		MI	19 (0.13)	7 (0.05)	< 5	< 5	97 (1.11)	< 5
		HF	30 (0.20)	11 (0.07)	< 5	< 5	372 (4.26)	5 (0.59)
		HS	< 5	< 5	< 5	< 5	20 (0.23)	< 5
		MP	9 (0.06)	< 5	< 5	< 5	20 (0.23)	< 5
	31 to 90 days	VTE	46 (0.30)	< 5	< 5	< 5	85 (0.97)	< 5
		DVT	21 (0.14)	< 5	< 5	< 5	57 (0.65)	< 5
		PE	28 (0.19)	< 5	< 5	< 5	38 (0.44)	< 5
		ATE	11 (0.07)	6 (0.04)	< 5	< 5	109 (1.25)	< 5
		IS	< 5	< 5	< 5	< 5	52 (0.60)	< 5
		TIA	< 5	< 5	< 5	< 5	27 (0.31)	< 5

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	< 5	< 5	< 5	38 (0.44)	< 5
		HF	14 (0.09)	5 (0.03)	< 5	< 5	142 (1.63)	< 5
		HS	< 5	< 5	< 5	< 5	7 (0.08)	< 5
		MP	8 (0.05)	< 5	< 5	< 5	9 (0.10)	< 5
	91 to 180 days	VTE	24 (0.16)	8 (0.05)	< 5	< 5	65 (0.74)	< 5
		DVT	11 (0.07)	6 (0.04)	< 5	< 5	36 (0.41)	< 5
		PE	14 (0.09)	< 5	< 5	< 5	33 (0.38)	< 5
		ATE	< 5	7 (0.05)	< 5	< 5	115 (1.32)	6 (0.71)
		IS	< 5	< 5	< 5	< 5	58 (0.66)	5 (0.59)
		TIA	< 5	< 5	< 5	< 5	22 (0.25)	< 5
		MI	< 5	< 5	< 5	< 5	38 (0.44)	< 5
		HF	10 (0.07)	< 5	< 5	< 5	122 (1.40)	< 5
		HS	< 5	< 5	< 5	< 5	9 (0.10)	< 5
		MP	< 5	< 5	< 5	< 5	8 (0.09)	< 5
	181 to 365 days	VTE	< 5	9 (0.06)	< 5	< 5	35 (0.40)	< 5
		DVT	< 5	< 5	< 5	< 5	21 (0.24)	< 5
		PE	< 5	6 (0.04)	< 5	< 5	16 (0.18)	< 5
		ATE	< 5	< 5	< 5	< 5	57 (0.65)	8 (0.95)
		IS	< 5	< 5	< 5	< 5	26 (0.30)	< 5
		TIA	< 5	< 5	< 5	< 5	12 (0.14)	< 5
		MI	< 5	< 5	< 5	< 5	20 (0.23)	< 5
		HF	< 5	< 5	< 5	< 5	64 (0.73)	5 (0.59)
		HS	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 4			N = 2,066,318	N = 542,670	N = 485,154	N = 147,744		
	0 to 30 days	VTE	346 (1.67)	8 (0.15)	38 (0.78)	6 (0.41)		
		DVT	56 (0.27)	< 5	6 (0.12)	< 5		
		PE	302 (1.46)	7 (0.13)	33 (0.68)	6 (0.41)		
		ATE	26 (0.13)	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.02)	< 5	< 5	< 5		
		MI	17 (0.08)	< 5	< 5	< 5		
		HF	28 (0.14)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	14 (0.07)	< 5	< 5	< 5		
	31 to 90 days	VTE	60 (0.29)	8 (0.15)	< 5	< 5		
		DVT	23 (0.11)	< 5	< 5	< 5		
		PE	40 (0.19)	5 (0.09)	< 5	< 5		
		ATE	13 (0.06)	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	9 (0.04)	< 5	< 5	< 5		
		HF	14 (0.07)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	7 (0.03)	< 5	< 5	< 5		
	91 to 180 days	VTE	29 (0.14)	< 5	< 5	< 5		
		DVT	10 (0.05)	< 5	< 5	< 5		
		PE	20 (0.10)	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	10 (0.05)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	181 to 365 days	VTE	< 5	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	< 5	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S30: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (ChAdOx1 vaccine). Only first outcome after COVID-19 captured.

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 344,687**	**N = 219,804**	**N = 168,972**	**N = 82,406**		
	0 to 30 days	**VTE**	77 (2.23)	41 (1.87)	7 (0.41)	< 5		
		DVT	16 (0.46)	12 (0.55)	< 5	< 5		
		PE	64 (1.86)	31 (1.41)	6 (0.36)	< 5		
		ATE	18 (0.52)	25 (1.14)	7 (0.41)	6 (0.73)		
		IS	6 (0.17)	< 5	< 5	< 5		
		TIA	< 5	7 (0.32)	< 5	< 5		
		MI	9 (0.26)	17 (0.77)	5 (0.30)	< 5		
		HF	42 (1.22)	72 (3.28)	9 (0.53)	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	31 to 90 days	**VTE**	12 (0.35)	14 (0.64)	< 5	< 5		
		DVT	5 (0.15)	8 (0.36)	< 5	< 5		
		PE	7 (0.20)	7 (0.32)	< 5	< 5		
		ATE	6 (0.17)	20 (0.91)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	9 (0.41)	< 5	< 5		
		MI	5 (0.15)	9 (0.41)	< 5	< 5		
		HF	26 (0.75)	39 (1.77)	< 5	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	91 to 180 days	**VTE**	6 (0.17)	15 (0.68)	< 5	< 5		
		DVT	< 5	5 (0.23)	< 5	< 5		
		PE	< 5	10 (0.45)	< 5	< 5		
		ATE	10 (0.29)	10 (0.45)	< 5	6 (0.73)		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.15)	6 (0.27)	< 5	< 5		
		MI	< 5	< 5	< 5	5 (0.61)		
		HF	28 (0.81)	35 (1.59)	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	< 5	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	9 (0.26)	9 (0.41)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	32 (0.93)	18 (0.82)	< 5	5 (0.61)		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
Cohort 2			N = 1,975,770	N = 969,262	N = 582,791	N = 302,999	N = 433,636	N = 323,204
	0 to 30 days	VTE	243 (1.23)	158 (1.63)	33 (0.57)	19 (0.63)	262 (6.04)	90 (2.78)
		DVT	42 (0.21)	47 (0.48)	8 (0.14)	< 5	65 (1.50)	45 (1.39)
		PE	206 (1.04)	120 (1.24)	26 (0.45)	15 (0.50)	218 (5.03)	55 (1.70)
		ATE	38 (0.19)	75 (0.77)	< 5	< 5	177 (4.08)	131 (4.05)
		IS	6 (0.03)	12 (0.12)	< 5	< 5	97 (2.24)	52 (1.61)
		TIA	7 (0.04)	13 (0.13)	< 5	< 5	17 (0.39)	23 (0.71)
		MI	25 (0.13)	52 (0.54)	< 5	< 5	67 (1.55)	62 (1.92)
		HF	46 (0.23)	103 (1.06)	5 (0.09)	11 (0.36)	379 (8.74)	123 (3.81)
		HS	5 (0.03)	< 5	< 5	< 5	21 (0.48)	7 (0.22)
		MP	11 (0.06)	6 (0.06)	< 5	< 5	15 (0.35)	10 (0.31)
	31 to 90 days	VTE	46 (0.23)	54 (0.56)	< 5	9 (0.30)	57 (1.31)	34 (1.05)
		DVT	24 (0.12)	25 (0.26)	< 5	5 (0.17)	30 (0.69)	23 (0.71)
		PE	25 (0.13)	31 (0.32)	< 5	< 5	32 (0.74)	16 (0.50)
		ATE	16 (0.08)	56 (0.58)	< 5	5 (0.17)	84 (1.94)	106 (3.28)
		IS	< 5	6 (0.06)	< 5	< 5	42 (0.97)	57 (1.76)
		TIA	< 5	24 (0.25)	< 5	< 5	23 (0.53)	24 (0.74)
		MI	11 (0.06)	28 (0.29)	< 5	< 5	26 (0.60)	27 (0.84)
		HF	24 (0.12)	65 (0.67)	< 5	7 (0.23)	134 (3.09)	61 (1.89)
		HS	< 5	< 5	< 5	< 5	7 (0.16)	7 (0.22)
		MP	6 (0.03)	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	22 (0.11)	28 (0.29)	< 5	< 5	52 (1.20)	21 (0.65)
		DVT	10 (0.05)	15 (0.15)	< 5	< 5	28 (0.65)	16 (0.50)
		PE	12 (0.06)	15 (0.15)	< 5	< 5	26 (0.60)	6 (0.19)

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	11 (0.06)	28 (0.29)	< 5	< 5	83 (1.91)	106 (3.28)
		IS	< 5	< 5	< 5	< 5	45 (1.04)	40 (1.24)
		TIA	7 (0.04)	13 (0.13)	< 5	< 5	20 (0.46)	32 (0.99)
		MI	5 (0.03)	15 (0.15)	< 5	< 5	21 (0.48)	37 (1.14)
		HF	16 (0.08)	41 (0.42)	< 5	< 5	105 (2.42)	54 (1.67)
		HS	< 5	< 5	< 5	< 5	7 (0.16)	< 5
		MP	< 5	< 5	< 5	< 5	7 (0.16)	< 5
	181 to 365 days	VTE	14 (0.07)	< 5	< 5	< 5	17 (0.39)	14 (0.43)
		DVT	< 5	< 5	< 5	< 5	9 (0.21)	12 (0.37)
		PE	10 (0.05)	< 5	< 5	< 5	9 (0.21)	< 5
		ATE	10 (0.05)	10 (0.10)	< 5	< 5	61 (1.41)	40 (1.24)
		IS	< 5	< 5	< 5	< 5	33 (0.76)	19 (0.59)
		TIA	< 5	< 5	< 5	< 5	18 (0.42)	12 (0.37)
		MI	7 (0.04)	6 (0.06)	< 5	< 5	12 (0.28)	13 (0.40)
		HF	17 (0.09)	22 (0.23)	< 5	< 5	70 (1.61)	25 (0.77)
		HS	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,493**	**N = 1,473,602**	**N = 418,184**	**N = 423,876**	**N = 873,400**	**N = 84,204**
	0 to 30 days	VTE	244 (1.62)	139 (0.94)	28 (0.67)	16 (0.38)	332 (3.80)	27 (3.21)
		DVT	44 (0.29)	41 (0.28)	6 (0.14)	< 5	95 (1.09)	9 (1.07)
		PE	209 (1.38)	101 (0.69)	23 (0.55)	12 (0.28)	266 (3.05)	22 (2.61)
		ATE	28 (0.19)	47 (0.32)	< 5	12 (0.28)	216 (2.47)	32 (3.80)
		IS	< 5	5 (0.03)	< 5	< 5	103 (1.18)	13 (1.54)
		TIA	5 (0.03)	16 (0.11)	< 5	< 5	22 (0.25)	5 (0.59)
		MI	19 (0.13)	26 (0.18)	< 5	9 (0.21)	97 (1.11)	16 (1.90)
		HF	30 (0.20)	28 (0.19)	< 5	< 5	372 (4.26)	51 (6.06)
		HS	< 5	< 5	< 5	< 5	20 (0.23)	< 5
		MP	9 (0.06)	6 (0.04)	< 5	< 5	20 (0.23)	6 (0.71)
	31 to 90 days	VTE	43 (0.28)	44 (0.30)	< 5	6 (0.14)	81 (0.93)	9 (1.07)
		DVT	20 (0.13)	26 (0.18)	< 5	< 5	54 (0.62)	< 5
		PE	26 (0.17)	18 (0.12)	< 5	< 5	36 (0.41)	6 (0.71)
		ATE	11 (0.07)	31 (0.21)	< 5	7 (0.17)	108 (1.24)	25 (2.97)
		IS	< 5	< 5	< 5	< 5	52 (0.60)	11 (1.31)
		TIA	< 5	13 (0.09)	< 5	< 5	27 (0.31)	< 5

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	16 (0.11)	< 5	< 5	37 (0.42)	10 (1.19)
		HF	14 (0.09)	23 (0.16)	< 5	< 5	135 (1.55)	25 (2.97)
		HS	< 5	< 5	< 5	< 5	7 (0.08)	< 5
		MP	8 (0.05)	7 (0.05)	< 5	< 5	9 (0.10)	< 5
	91 to 180 days	VTE	23 (0.15)	25 (0.17)	< 5	< 5	58 (0.66)	13 (1.54)
		DVT	10 (0.07)	15 (0.10)	< 5	< 5	33 (0.38)	7 (0.83)
		PE	14 (0.09)	13 (0.09)	< 5	< 5	29 (0.33)	7 (0.83)
		ATE	< 5	26 (0.18)	< 5	< 5	104 (1.19)	28 (3.33)
		IS	< 5	< 5	< 5	< 5	50 (0.57)	12 (1.43)
		TIA	< 5	8 (0.05)	< 5	< 5	20 (0.23)	10 (1.19)
		MI	< 5	17 (0.12)	< 5	< 5	36 (0.41)	8 (0.95)
		HF	10 (0.07)	10 (0.07)	< 5	< 5	111 (1.27)	12 (1.43)
		HS	< 5	< 5	< 5	< 5	9 (0.10)	< 5
		MP	< 5	< 5	< 5	< 5	8 (0.09)	< 5
	181 to 365 days	VTE	< 5	9 (0.06)	< 5	< 5	31 (0.35)	< 5
		DVT	< 5	< 5	< 5	< 5	18 (0.21)	< 5
		PE	< 5	6 (0.04)	< 5	< 5	15 (0.17)	< 5
		ATE	< 5	< 5	< 5	< 5	48 (0.55)	15 (1.78)
		IS	< 5	< 5	< 5	< 5	21 (0.24)	6 (0.71)
		TIA	< 5	< 5	< 5	< 5	11 (0.13)	< 5
		MI	< 5	< 5	< 5	< 5	17 (0.19)	8 (0.95)
		HF	< 5	< 5	< 5	< 5	55 (0.63)	6 (0.71)
		HS	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 4			N = 2,066,318	N = 542,670	N = 485,154	N = 147,744		
	0 to 30 days	VTE	346 (1.67)	27 (0.50)	38 (0.78)	8 (0.54)		
		DVT	56 (0.27)	7 (0.13)	6 (0.12)	< 5		
		PE	302 (1.46)	21 (0.39)	33 (0.68)	6 (0.41)		
		ATE	26 (0.13)	5 (0.09)	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	5 (0.02)	< 5	< 5	< 5		
		MI	17 (0.08)	< 5	< 5	< 5		
		HF	28 (0.14)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		

Cohort	Time window	Outcome	AURUM		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	14 (0.07)	< 5	< 5	< 5		
	31 to 90 days	VTE	57 (0.28)	14 (0.26)	< 5	< 5		
		DVT	22 (0.11)	8 (0.15)	< 5	< 5		
		PE	38 (0.18)	6 (0.11)	< 5	< 5		
		ATE	13 (0.06)	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	9 (0.04)	< 5	< 5	< 5		
		HF	14 (0.07)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	7 (0.03)	< 5	< 5	< 5		
	91 to 180 days	VTE	28 (0.14)	< 5	< 5	< 5		
		DVT	9 (0.04)	< 5	< 5	< 5		
		PE	20 (0.10)	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	10 (0.05)	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		
	181 to 365 days	VTE	< 5	< 5	< 5	< 5		
		DVT	< 5	< 5	< 5	< 5		
		PE	< 5	< 5	< 5	< 5		
		ATE	< 5	< 5	< 5	< 5		
		IS	< 5	< 5	< 5	< 5		
		TIA	< 5	< 5	< 5	< 5		
		MI	< 5	< 5	< 5	< 5		
		HF	< 5	< 5	< 5	< 5		
		HS	< 5	< 5	< 5	< 5		
		MP	< 5	< 5	< 5	< 5		

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S31: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (BNT162b2 vaccine).

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 348,052**	**N = 332,790**	**N = 24,073**	**N = 19,686**	**N = 169,459**	**N = 32,755**	**N = 223,960**	**N = 88,896**
	0 to 30 days	**VTE**	106 (3.05)	76 (2.28)	76 (31.57)	40 (20.32)	8 (0.47)	< 5	75 (3.35)	94 (10.57)
		DVT	21 (0.60)	15 (0.45)	14 (5.82)	13 (6.60)	< 5	< 5	20 (0.89)	29 (3.26)
		PE	90 (2.59)	64 (1.92)	64 (26.59)	28 (14.22)	6 (0.35)	< 5	60 (2.68)	75 (8.44)
		ATE	27 (0.78)	45 (1.35)	114 (47.36)	61 (30.99)	6 (0.35)	< 5	79 (3.53)	206 (23.17)
		IS	9 (0.26)	5 (0.15)	68 (28.25)	26 (13.21)	< 5	< 5	46 (2.05)	116 (13.05)
		TIA	5 (0.14)	11 (0.33)	22 (9.14)	15 (7.62)	< 5	< 5	7 (0.31)	41 (4.61)
		MI	14 (0.40)	29 (0.87)	34 (14.12)	27 (13.72)	< 5	< 5	28 (1.25)	61 (6.86)
		HF	59 (1.70)	126 (3.79)	393 (163.25)	231 (117.34)	9 (0.53)	< 5	299 (13.35)	634 (71.32)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.31)	14 (1.57)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	31 to 90 days	**VTE**	18 (0.52)	26 (0.78)	40 (16.62)	24 (12.19)	< 5	< 5	16 (0.71)	44 (4.95)
		DVT	9 (0.26)	7 (0.21)	19 (7.89)	14 (7.11)	< 5	< 5	10 (0.45)	29 (3.26)
		PE	9 (0.26)	19 (0.57)	24 (9.97)	10 (5.08)	< 5	< 5	6 (0.27)	19 (2.14)
		ATE	9 (0.26)	23 (0.69)	31 (12.88)	35 (17.78)	< 5	< 5	41 (1.83)	128 (14.40)
		IS	< 5	< 5	18 (7.48)	14 (7.11)	< 5	< 5	20 (0.89)	71 (7.99)
		TIA	5 (0.14)	10 (0.30)	7 (2.91)	14 (7.11)	< 5	< 5	13 (0.58)	32 (3.60)
		MI	5 (0.14)	11 (0.33)	11 (4.57)	10 (5.08)	< 5	< 5	11 (0.49)	29 (3.26)
		HF	32 (0.92)	71 (2.13)	151 (62.73)	141 (71.62)	< 5	< 5	87 (3.88)	296 (33.30)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	13 (1.46)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	**VTE**	10 (0.29)	6 (0.18)	19 (7.89)	27 (13.72)	< 5	< 5	21 (0.94)	40 (4.50)
		DVT	< 5	< 5	11 (4.57)	14 (7.11)	< 5	< 5	9 (0.40)	23 (2.59)
		PE	6 (0.17)	< 5	9 (3.74)	14 (7.11)	< 5	< 5	12 (0.54)	20 (2.25)
		ATE	15 (0.43)	18 (0.54)	31 (12.88)	40 (20.32)	< 5	< 5	31 (1.38)	110 (12.37)
		IS	< 5	6 (0.18)	16 (6.65)	17 (8.64)	< 5	< 5	17 (0.76)	59 (6.64)
		TIA	8 (0.23)	6 (0.18)	8 (3.32)	11 (5.59)	< 5	< 5	8 (0.36)	34 (3.82)
		MI	5 (0.14)	6 (0.18)	8 (3.32)	16 (8.13)	< 5	< 5	7 (0.31)	26 (2.92)
		HF	43 (1.24)	57 (1.71)	166 (68.96)	169 (85.85)	< 5	< 5	87 (3.88)	249 (28.01)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	14 (0.40)	10 (0.30)	43 (17.86)	31 (15.75)	< 5	< 5	10 (0.45)	12 (1.35)
		DVT	< 5	6 (0.18)	16 (6.65)	18 (9.14)	< 5	< 5	5 (0.22)	8 (0.90)
		PE	11 (0.32)	< 5	31 (12.88)	14 (7.11)	< 5	< 5	5 (0.22)	< 5
		ATE	15 (0.43)	14 (0.42)	57 (23.68)	62 (31.49)	< 5	< 5	41 (1.83)	52 (5.85)
		IS	< 5	< 5	32 (13.29)	30 (15.24)	< 5	< 5	20 (0.89)	33 (3.71)
		TIA	10 (0.29)	< 5	16 (6.65)	24 (12.19)	< 5	< 5	11 (0.49)	13 (1.46)
		MI	< 5	7 (0.21)	14 (5.82)	16 (8.13)	< 5	< 5	10 (0.45)	8 (0.90)
		HF	52 (1.49)	38 (1.14)	278 (115.48)	244 (123.95)	< 5	< 5	82 (3.66)	149 (16.76)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	9 (1.01)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 2			N = 1,976,163	N = 594,262			N = 584,309	N = 180,670	N = 433,111	N = 445,581
	0 to 30 days	VTE	240 (1.21)	62 (1.04)			29 (0.50)	5 (0.28)	259 (5.98)	265 (5.95)
		DVT	39 (0.20)	18 (0.30)			7 (0.12)	< 5	63 (1.45)	96 (2.15)
		PE	205 (1.04)	45 (0.76)			23 (0.39)	< 5	215 (4.96)	189 (4.24)
		ATE	39 (0.20)	29 (0.49)			< 5	< 5	172 (3.97)	489 (10.97)
		IS	7 (0.04)	< 5			< 5	< 5	94 (2.17)	267 (5.99)
		TIA	6 (0.03)	11 (0.19)			< 5	< 5	17 (0.39)	90 (2.02)
		MI	26 (0.13)	16 (0.27)			< 5	< 5	65 (1.50)	163 (3.66)
		HF	50 (0.25)	43 (0.72)			6 (0.10)	< 5	380 (8.77)	1,137 (25.52)
		HS	6 (0.03)	< 5			< 5	< 5	18 (0.42)	61 (1.37)
		MP	11 (0.06)	< 5			< 5	< 5	14 (0.32)	22 (0.49)
	31 to 90 days	VTE	49 (0.25)	21 (0.35)			< 5	< 5	60 (1.39)	128 (2.87)
		DVT	26 (0.13)	7 (0.12)			< 5	< 5	33 (0.76)	81 (1.82)
		PE	26 (0.13)	14 (0.24)			< 5	< 5	34 (0.79)	56 (1.26)
		ATE	17 (0.09)	36 (0.61)			< 5	< 5	86 (1.99)	313 (7.02)
		IS	< 5	5 (0.08)			< 5	< 5	43 (0.99)	167 (3.75)
		TIA	< 5	9 (0.15)			< 5	< 5	24 (0.55)	86 (1.93)
		MI	11 (0.06)	22 (0.37)			< 5	< 5	26 (0.60)	80 (1.80)
		HF	27 (0.14)	35 (0.59)			< 5	< 5	137 (3.16)	554 (12.43)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	19 (0.43)
		MP	6 (0.03)	< 5			< 5	< 5	< 5	< 5
	91 to 180 days	VTE	28 (0.14)	10 (0.17)			5 (0.09)	< 5	59 (1.36)	96 (2.15)
		DVT	12 (0.06)	6 (0.10)			< 5	< 5	32 (0.74)	59 (1.32)
		PE	16 (0.08)	5 (0.08)			< 5	< 5	30 (0.69)	45 (1.01)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	14 (0.07)	15 (0.25)			< 5	< 5	90 (2.08)	287 (6.44)
		IS	< 5	< 5			< 5	< 5	49 (1.13)	168 (3.77)
		TIA	7 (0.04)	5 (0.08)			< 5	< 5	21 (0.48)	76 (1.71)
		MI	8 (0.04)	10 (0.17)			< 5	< 5	23 (0.53)	63 (1.41)
		HF	19 (0.10)	25 (0.42)			< 5	< 5	110 (2.54)	472 (10.59)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	34 (0.76)
		MP	< 5	< 5			< 5	< 5	7 (0.16)	< 5
	181 to 365 days	VTE	10 (0.05)	8 (0.13)			< 5	< 5	16 (0.37)	40 (0.90)
		DVT	< 5	< 5			< 5	< 5	10 (0.23)	26 (0.58)
		PE	8 (0.04)	7 (0.12)			< 5	< 5	8 (0.18)	16 (0.36)
		ATE	12 (0.06)	7 (0.12)			< 5	< 5	59 (1.36)	123 (2.76)
		IS	< 5	< 5			< 5	< 5	29 (0.67)	58 (1.30)
		TIA	< 5	< 5			< 5	< 5	18 (0.42)	39 (0.88)
		MI	9 (0.05)	< 5			< 5	< 5	14 (0.32)	32 (0.72)
		HF	23 (0.12)	12 (0.20)			< 5	< 5	73 (1.69)	212 (4.76)
		HS	< 5	< 5			< 5	< 5	6 (0.14)	12 (0.27)
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,323**	**N = 54,102**			**N = 416,549**	**N = 36,748**	**N = 869,109**	**N = 706,435**
	0 to 30 days	VTE	244 (1.62)	< 5			28 (0.67)	< 5	321 (3.69)	114 (1.61)
		DVT	44 (0.29)	< 5			6 (0.14)	< 5	89 (1.02)	51 (0.72)
		PE	209 (1.38)	< 5			23 (0.55)	< 5	259 (2.98)	71 (1.01)
		ATE	29 (0.19)	< 5			< 5	< 5	211 (2.43)	197 (2.79)
		IS	< 5	< 5			< 5	< 5	102 (1.17)	95 (1.34)
		TIA	5 (0.03)	< 5			< 5	< 5	20 (0.23)	42 (0.59)
		MI	20 (0.13)	< 5			< 5	< 5	95 (1.09)	78 (1.10)
		HF	30 (0.20)	10 (1.85)			< 5	< 5	366 (4.21)	163 (2.31)
		HS	< 5	< 5			< 5	< 5	20 (0.23)	26 (0.37)
		MP	9 (0.06)	< 5			< 5	< 5	19 (0.22)	13 (0.18)
	31 to 90 days	VTE	44 (0.29)	< 5			< 5	< 5	84 (0.97)	65 (0.92)
		DVT	20 (0.13)	< 5			< 5	< 5	56 (0.64)	47 (0.67)
		PE	27 (0.18)	< 5			< 5	< 5	38 (0.44)	23 (0.33)
		ATE	11 (0.07)	< 5			< 5	< 5	107 (1.23)	144 (2.04)
		IS	< 5	< 5			< 5	< 5	53 (0.61)	55 (0.78)
		TIA	< 5	< 5			< 5	< 5	27 (0.31)	28 (0.40)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	< 5			< 5	< 5	35 (0.40)	69 (0.98)
		HF	15 (0.10)	< 5			< 5	< 5	139 (1.60)	111 (1.57)
		HS	< 5	< 5			< 5	< 5	7 (0.08)	14 (0.20)
		MP	8 (0.05)	< 5			< 5	< 5	8 (0.09)	9 (0.13)
	91 to 180 days	VTE	24 (0.16)	< 5			< 5	< 5	63 (0.72)	71 (1.01)
		DVT	11 (0.07)	< 5			< 5	< 5	35 (0.40)	45 (0.64)
		PE	14 (0.09)	< 5			< 5	< 5	32 (0.37)	27 (0.38)
		ATE	< 5	< 5			< 5	< 5	112 (1.29)	141 (2.00)
		IS	< 5	< 5			< 5	< 5	57 (0.66)	65 (0.92)
		TIA	< 5	< 5			< 5	< 5	22 (0.25)	29 (0.41)
		MI	< 5	< 5			< 5	< 5	36 (0.41)	53 (0.75)
		HF	10 (0.07)	< 5			< 5	< 5	121 (1.39)	100 (1.42)
		HS	< 5	< 5			< 5	< 5	9 (0.10)	10 (0.14)
		MP	< 5	< 5			< 5	< 5	7 (0.08)	9 (0.13)
	181 to 365 days	VTE	< 5	< 5			< 5	< 5	36 (0.41)	17 (0.24)
		DVT	< 5	< 5			< 5	< 5	22 (0.25)	14 (0.20)
		PE	< 5	< 5			< 5	< 5	16 (0.18)	< 5
		ATE	< 5	< 5			< 5	< 5	48 (0.55)	45 (0.64)
		IS	< 5	< 5			< 5	< 5	23 (0.26)	17 (0.24)
		TIA	< 5	< 5			< 5	< 5	9 (0.10)	7 (0.10)
		MI	< 5	< 5			< 5	< 5	17 (0.20)	24 (0.34)
		HF	5 (0.03)	< 5			< 5	< 5	61 (0.70)	31 (0.44)
		HS	< 5	< 5			< 5	< 5	< 5	< 5
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 4			**N = 2,014,161**	**N = 1,335,671**	**N = 147,553**	**N = 15,683**	**N = 465,326**	**N = 365,096**	**N = 1,068,043**	**N = 580,329**
	0 to 30 days	VTE	328 (1.63)	20 (0.15)	116 (7.86)	< 5	36 (0.77)	< 5	350 (3.28)	66 (1.14)
		DVT	52 (0.26)	9 (0.07)	22 (1.49)	< 5	6 (0.13)	< 5	107 (1.00)	35 (0.60)
		PE	287 (1.42)	11 (0.08)	97 (6.57)	< 5	31 (0.67)	< 5	271 (2.54)	35 (0.60)
		ATE	25 (0.12)	< 5	116 (7.86)	< 5	< 5	< 5	233 (2.18)	55 (0.95)
		IS	< 5	< 5	69 (4.68)	< 5	< 5	< 5	114 (1.07)	29 (0.50)
		TIA	6 (0.03)	< 5	18 (1.22)	< 5	< 5	< 5	28 (0.26)	7 (0.12)
		MI	15 (0.07)	< 5	38 (2.58)	< 5	< 5	< 5	97 (0.91)	20 (0.34)
		HF	26 (0.13)	< 5	362 (24.53)	8 (5.10)	< 5	< 5	369 (3.45)	44 (0.76)
		HS	< 5	< 5	5 (0.34)	< 5	< 5	< 5	24 (0.22)	7 (0.12)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	13 (0.06)	< 5	7 (0.47)	< 5	< 5	< 5	26 (0.24)	11 (0.19)
	31 to 90 days	VTE	56 (0.28)	7 (0.05)	54 (3.66)	< 5	< 5	< 5	93 (0.87)	33 (0.57)
		DVT	20 (0.10)	< 5	32 (2.17)	< 5	< 5	< 5	62 (0.58)	23 (0.40)
		PE	39 (0.19)	< 5	26 (1.76)	< 5	< 5	< 5	41 (0.38)	11 (0.19)
		ATE	13 (0.06)	< 5	46 (3.12)	< 5	< 5	< 5	115 (1.08)	51 (0.88)
		IS	< 5	< 5	24 (1.63)	< 5	< 5	< 5	51 (0.48)	21 (0.36)
		TIA	< 5	< 5	6 (0.41)	< 5	< 5	< 5	31 (0.29)	12 (0.21)
		MI	9 (0.04)	< 5	19 (1.29)	< 5	< 5	< 5	41 (0.38)	21 (0.36)
		HF	14 (0.07)	6 (0.04)	174 (11.79)	5 (3.19)	< 5	< 5	143 (1.34)	28 (0.48)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	10 (0.09)	5 (0.09)
		MP	7 (0.03)	< 5	5 (0.34)	< 5	< 5	< 5	12 (0.11)	8 (0.14)
	91 to 180 days	VTE	24 (0.12)	9 (0.07)	49 (3.32)	< 5	< 5	< 5	77 (0.72)	45 (0.78)
		DVT	11 (0.05)	7 (0.05)	31 (2.10)	< 5	< 5	< 5	42 (0.39)	28 (0.48)
		PE	14 (0.07)	< 5	19 (1.29)	< 5	< 5	< 5	38 (0.36)	20 (0.34)
		ATE	< 5	< 5	41 (2.78)	< 5	< 5	< 5	134 (1.25)	71 (1.22)
		IS	< 5	< 5	19 (1.29)	< 5	< 5	< 5	66 (0.62)	37 (0.64)
		TIA	< 5	< 5	12 (0.81)	< 5	< 5	< 5	31 (0.29)	14 (0.24)
		MI	< 5	< 5	14 (0.95)	< 5	< 5	< 5	40 (0.37)	20 (0.34)
		HF	9 (0.04)	< 5	208 (14.10)	< 5	< 5	< 5	145 (1.36)	35 (0.60)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	15 (0.14)	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	13 (0.12)	< 5
	181 to 365 days	VTE	< 5	< 5	77 (5.22)	5 (3.19)	< 5	< 5	52 (0.49)	10 (0.17)
		DVT	< 5	< 5	46 (3.12)	< 5	< 5	< 5	36 (0.34)	8 (0.14)
		PE	< 5	< 5	35 (2.37)	< 5	< 5	< 5	18 (0.17)	< 5
		ATE	< 5	< 5	72 (4.88)	< 5	< 5	< 5	52 (0.49)	21 (0.36)
		IS	< 5	< 5	33 (2.24)	< 5	< 5	< 5	28 (0.26)	11 (0.19)
		TIA	< 5	< 5	18 (1.22)	< 5	< 5	< 5	10 (0.09)	< 5
		MI	< 5	< 5	27 (1.83)	< 5	< 5	< 5	15 (0.14)	9 (0.16)
		HF	< 5	< 5	298 (20.20)	5 (3.19)	< 5	< 5	58 (0.54)	8 (0.14)
		HS	< 5	< 5	8 (0.54)	< 5	< 5	< 5	5 (0.05)	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	6 (0.06)	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S32: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (BNT162b2 vaccine). Follow-up ends at first vaccine dose after index date.

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 348,052**	**N = 332,790**	**N = 24,073**	**N = 19,686**	**N = 169,459**	**N = 32,755**	**N = 223,960**	**N = 88,896**
	0 to 30 days	**VTE**	106 (3.05)	31 (0.93)	76 (31.57)	< 5	8 (0.47)	< 5	75 (3.35)	< 5
		DVT	21 (0.60)	8 (0.24)	14 (5.82)	< 5	< 5	< 5	20 (0.89)	< 5
		PE	90 (2.59)	24 (0.72)	64 (26.59)	< 5	6 (0.35)	< 5	60 (2.68)	< 5
		ATE	27 (0.78)	18 (0.54)	114 (47.36)	< 5	6 (0.35)	< 5	79 (3.53)	5 (0.56)
		IS	9 (0.26)	< 5	68 (28.25)	< 5	< 5	< 5	46 (2.05)	< 5
		TIA	5 (0.14)	< 5	22 (9.14)	< 5	< 5	< 5	7 (0.31)	< 5
		MI	14 (0.40)	14 (0.42)	34 (14.12)	< 5	< 5	< 5	28 (1.25)	< 5
		HF	59 (1.70)	45 (1.35)	393 (163.25)	20 (10.16)	9 (0.53)	< 5	299 (13.35)	23 (2.59)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.31)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	31 to 90 days	**VTE**	18 (0.52)	15 (0.45)	40 (16.62)	< 5	< 5	< 5	16 (0.71)	< 5
		DVT	9 (0.26)	< 5	19 (7.89)	< 5	< 5	< 5	10 (0.45)	< 5
		PE	9 (0.26)	11 (0.33)	24 (9.97)	< 5	< 5	< 5	6 (0.27)	< 5
		ATE	9 (0.26)	8 (0.24)	31 (12.88)	< 5	< 5	< 5	41 (1.83)	5 (0.56)
		IS	< 5	< 5	18 (7.48)	< 5	< 5	< 5	20 (0.89)	< 5
		TIA	5 (0.14)	< 5	7 (2.91)	< 5	< 5	< 5	13 (0.58)	< 5
		MI	5 (0.14)	< 5	11 (4.57)	< 5	< 5	< 5	11 (0.49)	< 5
		HF	32 (0.92)	36 (1.08)	151 (62.73)	11 (5.59)	< 5	< 5	87 (3.88)	8 (0.90)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	**VTE**	10 (0.29)	< 5	19 (7.89)	< 5	< 5	< 5	21 (0.94)	< 5
		DVT	< 5	< 5	11 (4.57)	< 5	< 5	< 5	9 (0.40)	< 5
		PE	6 (0.17)	< 5	9 (3.74)	< 5	< 5	< 5	12 (0.54)	< 5
		ATE	15 (0.43)	10 (0.30)	31 (12.88)	< 5	< 5	< 5	31 (1.38)	< 5
		IS	< 5	< 5	16 (6.65)	< 5	< 5	< 5	17 (0.76)	< 5
		TIA	8 (0.23)	< 5	8 (3.32)	< 5	< 5	< 5	8 (0.36)	< 5
		MI	5 (0.14)	< 5	8 (3.32)	< 5	< 5	< 5	7 (0.31)	< 5
		HF	43 (1.24)	32 (0.96)	166 (68.96)	14 (7.11)	< 5	< 5	87 (3.88)	6 (0.67)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	14 (0.40)	7 (0.21)	43 (17.86)	6 (3.05)	< 5	< 5	10 (0.45)	< 5
		DVT	< 5	< 5	16 (6.65)	< 5	< 5	< 5	5 (0.22)	< 5
		PE	11 (0.32)	< 5	31 (12.88)	< 5	< 5	< 5	5 (0.22)	< 5
		ATE	15 (0.43)	11 (0.33)	57 (23.68)	10 (5.08)	< 5	< 5	41 (1.83)	8 (0.90)
		IS	< 5	< 5	32 (13.29)	9 (4.57)	< 5	< 5	20 (0.89)	5 (0.56)
		TIA	10 (0.29)	< 5	16 (6.65)	< 5	< 5	< 5	11 (0.49)	< 5
		MI	< 5	6 (0.18)	14 (5.82)	< 5	< 5	< 5	10 (0.45)	< 5
		HF	52 (1.49)	35 (1.05)	278 (115.48)	37 (18.80)	< 5	< 5	82 (3.66)	20 (2.25)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 2			**N = 1,976,163**	**N = 594,262**			**N = 584,309**	**N = 180,670**	**N = 433,111**	**N = 445,581**
	0 to 30 days	VTE	240 (1.21)	14 (0.24)			29 (0.50)	< 5	259 (5.98)	14 (0.31)
		DVT	39 (0.20)	6 (0.10)			7 (0.12)	< 5	63 (1.45)	< 5
		PE	205 (1.04)	8 (0.13)			23 (0.39)	< 5	215 (4.96)	12 (0.27)
		ATE	39 (0.20)	14 (0.24)			< 5	< 5	172 (3.97)	16 (0.36)
		IS	7 (0.04)	< 5			< 5	< 5	94 (2.17)	8 (0.18)
		TIA	6 (0.03)	< 5			< 5	< 5	17 (0.39)	< 5
		MI	26 (0.13)	8 (0.13)			< 5	< 5	65 (1.50)	5 (0.11)
		HF	50 (0.25)	15 (0.25)			6 (0.10)	< 5	380 (8.77)	56 (1.26)
		HS	6 (0.03)	< 5			< 5	< 5	18 (0.42)	< 5
		MP	11 (0.06)	< 5			< 5	< 5	14 (0.32)	< 5
	31 to 90 days	VTE	49 (0.25)	5 (0.08)			< 5	< 5	60 (1.39)	6 (0.13)
		DVT	26 (0.13)	< 5			< 5	< 5	33 (0.76)	< 5
		PE	26 (0.13)	< 5			< 5	< 5	34 (0.79)	< 5
		ATE	17 (0.09)	9 (0.15)			< 5	< 5	86 (1.99)	7 (0.16)
		IS	< 5	< 5			< 5	< 5	43 (0.99)	< 5
		TIA	< 5	< 5			< 5	< 5	24 (0.55)	< 5
		MI	11 (0.06)	< 5			< 5	< 5	26 (0.60)	< 5
		HF	27 (0.14)	17 (0.29)			< 5	< 5	137 (3.16)	19 (0.43)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	< 5
		MP	6 (0.03)	< 5			< 5	< 5	< 5	< 5
	91 to 180 days	VTE	28 (0.14)	< 5			5 (0.09)	< 5	59 (1.36)	< 5
		DVT	12 (0.06)	< 5			< 5	< 5	32 (0.74)	< 5
		PE	16 (0.08)	< 5			< 5	< 5	30 (0.69)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	14 (0.07)	5 (0.08)			< 5	< 5	90 (2.08)	10 (0.22)
		IS	< 5	< 5			< 5	< 5	49 (1.13)	5 (0.11)
		TIA	7 (0.04)	< 5			< 5	< 5	21 (0.48)	< 5
		MI	8 (0.04)	< 5			< 5	< 5	23 (0.53)	< 5
		HF	19 (0.10)	9 (0.15)			< 5	< 5	110 (2.54)	25 (0.56)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	< 5
		MP	< 5	< 5			< 5	< 5	7 (0.16)	< 5
	181 to 365 days	VTE	10 (0.05)	7 (0.12)			< 5	< 5	16 (0.37)	10 (0.22)
		DVT	< 5	< 5			< 5	< 5	10 (0.23)	6 (0.13)
		PE	8 (0.04)	5 (0.08)			< 5	< 5	8 (0.18)	< 5
		ATE	12 (0.06)	6 (0.10)			< 5	< 5	59 (1.36)	19 (0.43)
		IS	< 5	< 5			< 5	< 5	29 (0.67)	9 (0.20)
		TIA	< 5	< 5			< 5	< 5	18 (0.42)	5 (0.11)
		MI	9 (0.05)	< 5			< 5	< 5	14 (0.32)	5 (0.11)
		HF	23 (0.12)	11 (0.19)			< 5	< 5	73 (1.69)	27 (0.61)
		HS	< 5	< 5			< 5	< 5	6 (0.14)	< 5
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,323**	**N = 54,102**			**N = 416,549**	**N = 36,748**	**N = 869,109**	**N = 706,435**
	0 to 30 days	VTE	244 (1.62)	< 5			28 (0.67)	< 5	321 (3.69)	< 5
		DVT	44 (0.29)	< 5			6 (0.14)	< 5	89 (1.02)	< 5
		PE	209 (1.38)	< 5			23 (0.55)	< 5	259 (2.98)	< 5
		ATE	29 (0.19)	< 5			< 5	< 5	211 (2.43)	< 5
		IS	< 5	< 5			< 5	< 5	102 (1.17)	< 5
		TIA	5 (0.03)	< 5			< 5	< 5	20 (0.23)	< 5
		MI	20 (0.13)	< 5			< 5	< 5	95 (1.09)	< 5
		HF	30 (0.20)	7 (1.29)			< 5	< 5	366 (4.21)	5 (0.07)
		HS	< 5	< 5			< 5	< 5	20 (0.23)	< 5
		MP	9 (0.06)	< 5			< 5	< 5	19 (0.22)	< 5
	31 to 90 days	VTE	44 (0.29)	< 5			< 5	< 5	84 (0.97)	< 5
		DVT	20 (0.13)	< 5			< 5	< 5	56 (0.64)	< 5
		PE	27 (0.18)	< 5			< 5	< 5	38 (0.44)	< 5
		ATE	11 (0.07)	< 5			< 5	< 5	107 (1.23)	< 5
		IS	< 5	< 5			< 5	< 5	53 (0.61)	< 5
		TIA	< 5	< 5			< 5	< 5	27 (0.31)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	< 5			< 5	< 5	35 (0.40)	< 5
		HF	15 (0.10)	< 5			< 5	< 5	139 (1.60)	< 5
		HS	< 5	< 5			< 5	< 5	7 (0.08)	< 5
		MP	8 (0.05)	< 5			< 5	< 5	8 (0.09)	< 5
	91 to 180 days	VTE	24 (0.16)	< 5			< 5	< 5	63 (0.72)	< 5
		DVT	11 (0.07)	< 5			< 5	< 5	35 (0.40)	< 5
		PE	14 (0.09)	< 5			< 5	< 5	32 (0.37)	< 5
		ATE	< 5	< 5			< 5	< 5	112 (1.29)	6 (0.08)
		IS	< 5	< 5			< 5	< 5	57 (0.66)	< 5
		TIA	< 5	< 5			< 5	< 5	22 (0.25)	< 5
		MI	< 5	< 5			< 5	< 5	36 (0.41)	< 5
		HF	10 (0.07)	< 5			< 5	< 5	121 (1.39)	< 5
		HS	< 5	< 5			< 5	< 5	9 (0.10)	< 5
		MP	< 5	< 5			< 5	< 5	7 (0.08)	< 5
	181 to 365 days	VTE	< 5	< 5			< 5	< 5	36 (0.41)	< 5
		DVT	< 5	< 5			< 5	< 5	22 (0.25)	< 5
		PE	< 5	< 5			< 5	< 5	16 (0.18)	< 5
		ATE	< 5	< 5			< 5	< 5	48 (0.55)	6 (0.08)
		IS	< 5	< 5			< 5	< 5	23 (0.26)	< 5
		TIA	< 5	< 5			< 5	< 5	9 (0.10)	< 5
		MI	< 5	< 5			< 5	< 5	17 (0.20)	< 5
		HF	5 (0.03)	< 5			< 5	< 5	61 (0.70)	< 5
		HS	< 5	< 5			< 5	< 5	< 5	< 5
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 4			N = 2,014,161	N = 1,335,671	N = 147,553	N = 15,683	N = 465,326	N = 365,096	N = 1,068,043	N = 580,329
	0 to 30 days	VTE	328 (1.63)	9 (0.07)	116 (7.86)	< 5	36 (0.77)	< 5	350 (3.28)	18 (0.31)
		DVT	52 (0.26)	5 (0.04)	22 (1.49)	< 5	6 (0.13)	< 5	107 (1.00)	8 (0.14)
		PE	287 (1.42)	< 5	97 (6.57)	< 5	31 (0.67)	< 5	271 (2.54)	10 (0.17)
		ATE	25 (0.12)	< 5	116 (7.86)	< 5	< 5	< 5	233 (2.18)	9 (0.16)
		IS	< 5	< 5	69 (4.68)	< 5	< 5	< 5	114 (1.07)	6 (0.10)
		TIA	6 (0.03)	< 5	18 (1.22)	< 5	< 5	< 5	28 (0.26)	< 5
		MI	15 (0.07)	< 5	38 (2.58)	< 5	< 5	< 5	97 (0.91)	< 5
		HF	26 (0.13)	< 5	362 (24.53)	6 (3.83)	< 5	< 5	369 (3.45)	7 (0.12)
		HS	< 5	< 5	5 (0.34)	< 5	< 5	< 5	24 (0.22)	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	13 (0.06)	< 5	7 (0.47)	< 5	< 5	< 5	26 (0.24)	< 5
	31 to 90 days	**VTE**	56 (0.28)	< 5	54 (3.66)	< 5	< 5	< 5	93 (0.87)	6 (0.10)
		DVT	20 (0.10)	< 5	32 (2.17)	< 5	< 5	< 5	62 (0.58)	< 5
		PE	39 (0.19)	< 5	26 (1.76)	< 5	< 5	< 5	41 (0.38)	< 5
		ATE	13 (0.06)	< 5	46 (3.12)	< 5	< 5	< 5	115 (1.08)	< 5
		IS	< 5	< 5	24 (1.63)	< 5	< 5	< 5	51 (0.48)	< 5
		TIA	< 5	< 5	6 (0.41)	< 5	< 5	< 5	31 (0.29)	< 5
		MI	9 (0.04)	< 5	19 (1.29)	< 5	< 5	< 5	41 (0.38)	< 5
		HF	14 (0.07)	6 (0.04)	174 (11.79)	< 5	< 5	< 5	143 (1.34)	< 5
		HS	< 5	< 5	< 5	< 5	< 5	< 5	10 (0.09)	< 5
		MP	7 (0.03)	< 5	5 (0.34)	< 5	< 5	< 5	12 (0.11)	< 5
	91 to 180 days	**VTE**	24 (0.12)	8 (0.06)	49 (3.32)	< 5	< 5	< 5	77 (0.72)	< 5
		DVT	11 (0.05)	7 (0.05)	31 (2.10)	< 5	< 5	< 5	42 (0.39)	< 5
		PE	14 (0.07)	< 5	19 (1.29)	< 5	< 5	< 5	38 (0.36)	< 5
		ATE	< 5	< 5	41 (2.78)	< 5	< 5	< 5	134 (1.25)	9 (0.16)
		IS	< 5	< 5	19 (1.29)	< 5	< 5	< 5	66 (0.62)	7 (0.12)
		TIA	< 5	< 5	12 (0.81)	< 5	< 5	< 5	31 (0.29)	< 5
		MI	< 5	< 5	14 (0.95)	< 5	< 5	< 5	40 (0.37)	< 5
		HF	9 (0.04)	< 5	208 (14.10)	< 5	< 5	< 5	145 (1.36)	9 (0.16)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	15 (0.14)	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	13 (0.12)	< 5
	181 to 365 days	**VTE**	< 5	< 5	77 (5.22)	5 (3.19)	< 5	< 5	52 (0.49)	7 (0.12)
		DVT	< 5	< 5	46 (3.12)	< 5	< 5	< 5	36 (0.34)	5 (0.09)
		PE	< 5	< 5	35 (2.37)	< 5	< 5	< 5	18 (0.17)	< 5
		ATE	< 5	< 5	72 (4.88)	< 5	< 5	< 5	52 (0.49)	15 (0.26)
		IS	< 5	< 5	33 (2.24)	< 5	< 5	< 5	28 (0.26)	10 (0.17)
		TIA	< 5	< 5	18 (1.22)	< 5	< 5	< 5	10 (0.09)	< 5
		MI	< 5	< 5	27 (1.83)	< 5	< 5	< 5	15 (0.14)	5 (0.09)
		HF	< 5	< 5	298 (20.20)	< 5	< 5	< 5	58 (0.54)	< 5
		HS	< 5	< 5	8 (0.54)	< 5	< 5	< 5	5 (0.05)	< 5
		MP	< 5	< 5	5 (0.34)	< 5	< 5	< 5	6 (0.06)	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S33: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by exposure status (BNT162b2 vaccine). Only first outcome after COVID-19 captured.

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
Cohort 1			**N = 348,052**	**N = 332,790**	**N = 24,073**	**N = 19,686**	**N = 169,459**	**N = 32,755**	**N = 223,960**	**N = 88,896**
	0 to 30 days	**VTE**	106 (3.05)	76 (2.28)	76 (31.57)	40 (20.32)	8 (0.47)	< 5	75 (3.35)	94 (10.57)
		DVT	21 (0.60)	15 (0.45)	14 (5.82)	13 (6.60)	< 5	< 5	20 (0.89)	29 (3.26)
		PE	90 (2.59)	64 (1.92)	64 (26.59)	28 (14.22)	6 (0.35)	< 5	60 (2.68)	75 (8.44)
		ATE	27 (0.78)	45 (1.35)	114 (47.36)	61 (30.99)	6 (0.35)	< 5	79 (3.53)	206 (23.17)
		IS	9 (0.26)	5 (0.15)	68 (28.25)	26 (13.21)	< 5	< 5	46 (2.05)	116 (13.05)
		TIA	5 (0.14)	11 (0.33)	22 (9.14)	15 (7.62)	< 5	< 5	7 (0.31)	41 (4.61)
		MI	14 (0.40)	29 (0.87)	34 (14.12)	27 (13.72)	< 5	< 5	28 (1.25)	61 (6.86)
		HF	59 (1.70)	126 (3.79)	393 (163.25)	231 (117.34)	9 (0.53)	< 5	299 (13.35)	634 (71.32)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.31)	14 (1.57)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	31 to 90 days	**VTE**	18 (0.52)	25 (0.75)	37 (15.37)	23 (11.68)	< 5	< 5	15 (0.67)	43 (4.84)
		DVT	9 (0.26)	7 (0.21)	18 (7.48)	13 (6.60)	< 5	< 5	9 (0.40)	28 (3.15)
		PE	9 (0.26)	18 (0.54)	22 (9.14)	10 (5.08)	< 5	< 5	6 (0.27)	19 (2.14)
		ATE	9 (0.26)	23 (0.69)	27 (11.22)	33 (16.76)	< 5	< 5	39 (1.74)	125 (14.06)
		IS	< 5	< 5	15 (6.23)	13 (6.60)	< 5	< 5	20 (0.89)	69 (7.76)
		TIA	5 (0.14)	10 (0.30)	7 (2.91)	14 (7.11)	< 5	< 5	13 (0.58)	32 (3.60)
		MI	5 (0.14)	11 (0.33)	9 (3.74)	8 (4.06)	< 5	< 5	9 (0.40)	28 (3.15)
		HF	32 (0.92)	70 (2.10)	136 (56.49)	135 (68.58)	< 5	< 5	83 (3.71)	288 (32.40)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	12 (1.35)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
	91 to 180 days	**VTE**	10 (0.29)	< 5	14 (5.82)	22 (11.18)	< 5	< 5	19 (0.85)	35 (3.94)
		DVT	< 5	< 5	6 (2.49)	11 (5.59)	< 5	< 5	7 (0.31)	19 (2.14)
		PE	6 (0.17)	< 5	9 (3.74)	12 (6.10)	< 5	< 5	12 (0.54)	18 (2.02)
		ATE	15 (0.43)	18 (0.54)	29 (12.05)	35 (17.78)	< 5	< 5	26 (1.16)	102 (11.47)
		IS	< 5	6 (0.18)	14 (5.82)	14 (7.11)	< 5	< 5	14 (0.63)	56 (6.30)
		TIA	8 (0.23)	6 (0.18)	8 (3.32)	10 (5.08)	< 5	< 5	7 (0.31)	33 (3.71)
		MI	5 (0.14)	6 (0.18)	8 (3.32)	15 (7.62)	< 5	< 5	6 (0.27)	22 (2.47)
		HF	40 (1.15)	54 (1.62)	147 (61.06)	159 (80.77)	< 5	< 5	81 (3.62)	226 (25.42)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
	181 to 365 days	VTE	11 (0.32)	10 (0.30)	27 (11.22)	24 (12.19)	< 5	< 5	5 (0.22)	10 (1.12)
		DVT	< 5	6 (0.18)	10 (4.15)	14 (7.11)	< 5	< 5	< 5	7 (0.79)
		PE	9 (0.26)	< 5	21 (8.72)	11 (5.59)	< 5	< 5	< 5	< 5
		ATE	14 (0.40)	14 (0.42)	47 (19.52)	58 (29.46)	< 5	< 5	34 (1.52)	38 (4.27)
		IS	< 5	< 5	26 (10.80)	27 (13.72)	< 5	< 5	17 (0.76)	23 (2.59)
		TIA	9 (0.26)	< 5	15 (6.23)	24 (12.19)	< 5	< 5	10 (0.45)	9 (1.01)
		MI	< 5	7 (0.21)	10 (4.15)	12 (6.10)	< 5	< 5	7 (0.31)	7 (0.79)
		HF	47 (1.35)	35 (1.05)	226 (93.88)	223 (113.28)	< 5	< 5	70 (3.13)	117 (13.16)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	< 5	7 (0.79)
		MP	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cohort 2			N = 1,976,163	N = 594,262			N = 584,309	N = 180,670	N = 433,111	N = 445,581
	0 to 30 days	VTE	240 (1.21)	62 (1.04)			29 (0.50)	5 (0.28)	259 (5.98)	265 (5.95)
		DVT	39 (0.20)	18 (0.30)			7 (0.12)	< 5	63 (1.45)	96 (2.15)
		PE	205 (1.04)	45 (0.76)			23 (0.39)	< 5	215 (4.96)	189 (4.24)
		ATE	39 (0.20)	29 (0.49)			< 5	< 5	172 (3.97)	489 (10.97)
		IS	7 (0.04)	< 5			< 5	< 5	94 (2.17)	267 (5.99)
		TIA	6 (0.03)	11 (0.19)			< 5	< 5	17 (0.39)	90 (2.02)
		MI	26 (0.13)	16 (0.27)			< 5	< 5	65 (1.50)	163 (3.66)
		HF	50 (0.25)	43 (0.72)			6 (0.10)	< 5	380 (8.77)	1,137 (25.52)
		HS	6 (0.03)	< 5			< 5	< 5	18 (0.42)	61 (1.37)
		MP	11 (0.06)	< 5			< 5	< 5	14 (0.32)	22 (0.49)
	31 to 90 days	VTE	46 (0.23)	21 (0.35)			< 5	< 5	57 (1.32)	123 (2.76)
		DVT	25 (0.13)	7 (0.12)			< 5	< 5	31 (0.72)	77 (1.73)
		PE	24 (0.12)	14 (0.24)			< 5	< 5	32 (0.74)	55 (1.23)
		ATE	17 (0.09)	36 (0.61)			< 5	< 5	84 (1.94)	307 (6.89)
		IS	< 5	5 (0.08)			< 5	< 5	43 (0.99)	162 (3.64)
		TIA	< 5	9 (0.15)			< 5	< 5	24 (0.55)	85 (1.91)
		MI	11 (0.06)	22 (0.37)			< 5	< 5	24 (0.55)	79 (1.77)
		HF	27 (0.14)	34 (0.57)			< 5	< 5	131 (3.02)	534 (11.98)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	19 (0.43)
		MP	6 (0.03)	< 5			< 5	< 5	< 5	< 5
	91 to 180 days	VTE	25 (0.13)	9 (0.15)			5 (0.09)	< 5	53 (1.22)	84 (1.89)
		DVT	11 (0.06)	6 (0.10)			< 5	< 5	29 (0.67)	52 (1.17)
		PE	14 (0.07)	< 5			< 5	< 5	27 (0.62)	39 (0.88)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		ATE	13 (0.07)	15 (0.25)			< 5	< 5	80 (1.85)	265 (5.95)
		IS	< 5	< 5			< 5	< 5	43 (0.99)	148 (3.32)
		TIA	7 (0.04)	5 (0.08)			< 5	< 5	19 (0.44)	72 (1.62)
		MI	7 (0.04)	10 (0.17)			< 5	< 5	21 (0.48)	62 (1.39)
		HF	19 (0.10)	24 (0.40)			< 5	< 5	101 (2.33)	438 (9.83)
		HS	< 5	< 5			< 5	< 5	7 (0.16)	33 (0.74)
		MP	< 5	< 5			< 5	< 5	7 (0.16)	< 5
	181 to 365 days	VTE	9 (0.05)	8 (0.13)			< 5	< 5	15 (0.35)	30 (0.67)
		DVT	< 5	< 5			< 5	< 5	9 (0.21)	20 (0.45)
		PE	7 (0.04)	7 (0.12)			< 5	< 5	8 (0.18)	12 (0.27)
		ATE	9 (0.05)	7 (0.12)			< 5	< 5	50 (1.15)	103 (2.31)
		IS	< 5	< 5			< 5	< 5	25 (0.58)	51 (1.14)
		TIA	< 5	< 5			< 5	< 5	16 (0.37)	29 (0.65)
		MI	7 (0.04)	< 5			< 5	< 5	11 (0.25)	29 (0.65)
		HF	22 (0.11)	12 (0.20)			< 5	< 5	63 (1.45)	178 (3.99)
		HS	< 5	< 5			< 5	< 5	5 (0.12)	9 (0.20)
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 3			**N = 1,510,323**	**N = 54,102**			**N = 416,549**	**N = 36,748**	**N = 869,109**	**N = 706,435**
	0 to 30 days	VTE	244 (1.62)	< 5			28 (0.67)	< 5	321 (3.69)	114 (1.61)
		DVT	44 (0.29)	< 5			6 (0.14)	< 5	89 (1.02)	51 (0.72)
		PE	209 (1.38)	< 5			23 (0.55)	< 5	259 (2.98)	71 (1.01)
		ATE	29 (0.19)	< 5			< 5	< 5	211 (2.43)	197 (2.79)
		IS	< 5	< 5			< 5	< 5	102 (1.17)	95 (1.34)
		TIA	5 (0.03)	< 5			< 5	< 5	20 (0.23)	42 (0.59)
		MI	20 (0.13)	< 5			< 5	< 5	95 (1.09)	78 (1.10)
		HF	30 (0.20)	10 (1.85)			< 5	< 5	366 (4.21)	163 (2.31)
		HS	< 5	< 5			< 5	< 5	20 (0.23)	26 (0.37)
		MP	9 (0.06)	< 5			< 5	< 5	19 (0.22)	13 (0.18)
	31 to 90 days	VTE	41 (0.27)	< 5			< 5	< 5	80 (0.92)	63 (0.89)
		DVT	19 (0.13)	< 5			< 5	< 5	53 (0.61)	47 (0.67)
		PE	25 (0.17)	< 5			< 5	< 5	36 (0.41)	21 (0.30)
		ATE	11 (0.07)	< 5			< 5	< 5	106 (1.22)	142 (2.01)
		IS	< 5	< 5			< 5	< 5	53 (0.61)	54 (0.76)
		TIA	< 5	< 5			< 5	< 5	27 (0.31)	27 (0.38)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MI	9 (0.06)	< 5			< 5	< 5	34 (0.39)	69 (0.98)
		HF	15 (0.10)	< 5			< 5	< 5	132 (1.52)	108 (1.53)
		HS	< 5	< 5			< 5	< 5	7 (0.08)	14 (0.20)
		MP	8 (0.05)	< 5			< 5	< 5	8 (0.09)	9 (0.13)
	91 to 180 days	VTE	23 (0.15)	< 5			< 5	< 5	56 (0.64)	67 (0.95)
		DVT	10 (0.07)	< 5			< 5	< 5	32 (0.37)	43 (0.61)
		PE	14 (0.09)	< 5			< 5	< 5	28 (0.32)	25 (0.35)
		ATE	< 5	< 5			< 5	< 5	101 (1.16)	137 (1.94)
		IS	< 5	< 5			< 5	< 5	49 (0.56)	62 (0.88)
		TIA	< 5	< 5			< 5	< 5	20 (0.23)	28 (0.40)
		MI	< 5	< 5			< 5	< 5	34 (0.39)	53 (0.75)
		HF	10 (0.07)	< 5			< 5	< 5	110 (1.27)	97 (1.37)
		HS	< 5	< 5			< 5	< 5	9 (0.10)	10 (0.14)
		MP	< 5	< 5			< 5	< 5	7 (0.08)	9 (0.13)
	181 to 365 days	VTE	< 5	< 5			< 5	< 5	33 (0.38)	13 (0.18)
		DVT	< 5	< 5			< 5	< 5	20 (0.23)	11 (0.16)
		PE	< 5	< 5			< 5	< 5	15 (0.17)	< 5
		ATE	< 5	< 5			< 5	< 5	39 (0.45)	36 (0.51)
		IS	< 5	< 5			< 5	< 5	18 (0.21)	13 (0.18)
		TIA	< 5	< 5			< 5	< 5	8 (0.09)	5 (0.07)
		MI	< 5	< 5			< 5	< 5	14 (0.16)	21 (0.30)
		HF	5 (0.03)	< 5			< 5	< 5	53 (0.61)	25 (0.35)
		HS	< 5	< 5			< 5	< 5	< 5	< 5
		MP	< 5	< 5			< 5	< 5	< 5	< 5
Cohort 4			**N = 2,014,161**	**N = 1,335,671**	**N = 147,553**	**N = 15,683**	**N = 465,326**	**N = 365,096**	**N = 1,068,043**	**N = 580,329**
	0 to 30 days	VTE	328 (1.63)	20 (0.15)	116 (7.86)	< 5	36 (0.77)	< 5	350 (3.28)	66 (1.14)
		DVT	52 (0.26)	9 (0.07)	22 (1.49)	< 5	6 (0.13)	< 5	107 (1.00)	35 (0.60)
		PE	287 (1.42)	11 (0.08)	97 (6.57)	< 5	31 (0.67)	< 5	271 (2.54)	35 (0.60)
		ATE	25 (0.12)	< 5	116 (7.86)	< 5	< 5	< 5	233 (2.18)	55 (0.95)
		IS	< 5	< 5	69 (4.68)	< 5	< 5	< 5	114 (1.07)	29 (0.50)
		TIA	6 (0.03)	< 5	18 (1.22)	< 5	< 5	< 5	28 (0.26)	7 (0.12)
		MI	15 (0.07)	< 5	38 (2.58)	< 5	< 5	< 5	97 (0.91)	20 (0.34)
		HF	26 (0.13)	< 5	362 (24.53)	8 (5.10)	< 5	< 5	369 (3.45)	44 (0.76)
		HS	< 5	< 5	5 (0.34)	< 5	< 5	< 5	24 (0.22)	7 (0.12)

Cohort	Time window	Outcome	AURUM		CORIVA		GOLD		SIDIAP	
			Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated
		MP	13 (0.06)	< 5	7 (0.47)	< 5	< 5	< 5	26 (0.24)	11 (0.19)
	31 to 90 days	VTE	53 (0.26)	7 (0.05)	49 (3.32)	< 5	< 5	< 5	88 (0.82)	32 (0.55)
		DVT	19 (0.09)	< 5	29 (1.97)	< 5	< 5	< 5	58 (0.54)	23 (0.40)
		PE	37 (0.18)	< 5	23 (1.56)	< 5	< 5	< 5	39 (0.37)	10 (0.17)
		ATE	13 (0.06)	< 5	43 (2.91)	< 5	< 5	< 5	114 (1.07)	51 (0.88)
		IS	< 5	< 5	22 (1.49)	< 5	< 5	< 5	51 (0.48)	21 (0.36)
		TIA	< 5	< 5	6 (0.41)	< 5	< 5	< 5	31 (0.29)	12 (0.21)
		MI	9 (0.04)	< 5	17 (1.15)	< 5	< 5	< 5	40 (0.37)	21 (0.36)
		HF	14 (0.07)	6 (0.04)	160 (10.84)	5 (3.19)	< 5	< 5	136 (1.27)	28 (0.48)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	10 (0.09)	5 (0.09)
		MP	7 (0.03)	< 5	< 5	< 5	< 5	< 5	12 (0.11)	8 (0.14)
	91 to 180 days	VTE	23 (0.11)	9 (0.07)	40 (2.71)	< 5	< 5	< 5	71 (0.66)	40 (0.69)
		DVT	10 (0.05)	7 (0.05)	25 (1.69)	< 5	< 5	< 5	40 (0.37)	25 (0.43)
		PE	14 (0.07)	< 5	16 (1.08)	< 5	< 5	< 5	34 (0.32)	18 (0.31)
		ATE	< 5	< 5	38 (2.58)	< 5	< 5	< 5	122 (1.14)	68 (1.17)
		IS	< 5	< 5	18 (1.22)	< 5	< 5	< 5	58 (0.54)	34 (0.59)
		TIA	< 5	< 5	12 (0.81)	< 5	< 5	< 5	28 (0.26)	14 (0.24)
		MI	< 5	< 5	12 (0.81)	< 5	< 5	< 5	38 (0.36)	20 (0.34)
		HF	9 (0.04)	< 5	187 (12.67)	< 5	< 5	< 5	132 (1.24)	35 (0.60)
		HS	< 5	< 5	< 5	< 5	< 5	< 5	14 (0.13)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	13 (0.12)	< 5
	181 to 365 days	VTE	< 5	< 5	53 (3.59)	< 5	< 5	< 5	44 (0.41)	7 (0.12)
		DVT	< 5	< 5	33 (2.24)	< 5	< 5	< 5	31 (0.29)	6 (0.10)
		PE	< 5	< 5	23 (1.56)	< 5	< 5	< 5	15 (0.14)	< 5
		ATE	< 5	< 5	63 (4.27)	< 5	< 5	< 5	41 (0.38)	9 (0.16)
		IS	< 5	< 5	29 (1.97)	< 5	< 5	< 5	20 (0.19)	6 (0.10)
		TIA	< 5	< 5	18 (1.22)	< 5	< 5	< 5	9 (0.08)	< 5
		MI	< 5	< 5	21 (1.42)	< 5	< 5	< 5	13 (0.12)	< 5
		HF	< 5	< 5	240 (16.27)	< 5	< 5	< 5	49 (0.46)	5 (0.09)
		HS	< 5	< 5	8 (0.54)	< 5	< 5	< 5	5 (0.05)	< 5
		MP	< 5	< 5	< 5	< 5	< 5	< 5	6 (0.06)	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S34: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by vaccine (BNT162b2 - ChAdOx1).

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
Cohort 1			N = 332,790	N = 219,804	N = 32,755	N = 82,406
	0 to 30 days	VTE	76 (2.28)	41 (1.87)	< 5	< 5
		DVT	15 (0.45)	12 (0.55)	< 5	< 5
		PE	64 (1.92)	31 (1.41)	< 5	< 5
		ATE	45 (1.35)	25 (1.14)	< 5	6 (0.73)
		IS	5 (0.15)	< 5	< 5	< 5
		TIA	11 (0.33)	7 (0.32)	< 5	< 5
		MI	29 (0.87)	17 (0.77)	< 5	< 5
		HF	126 (3.79)	72 (3.28)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	26 (0.78)	14 (0.64)	< 5	< 5
		DVT	7 (0.21)	8 (0.36)	< 5	< 5
		PE	19 (0.57)	7 (0.32)	< 5	< 5
		ATE	23 (0.69)	20 (0.91)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	10 (0.30)	9 (0.41)	< 5	< 5
		MI	11 (0.33)	9 (0.41)	< 5	< 5
		HF	71 (2.13)	42 (1.91)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	6 (0.18)	15 (0.68)	< 5	< 5
		DVT	< 5	5 (0.23)	< 5	< 5
		PE	< 5	10 (0.45)	< 5	< 5
		ATE	18 (0.54)	10 (0.45)	< 5	6 (0.73)
		IS	6 (0.18)	< 5	< 5	< 5
		TIA	6 (0.18)	6 (0.27)	< 5	< 5
		MI	6 (0.18)	< 5	< 5	5 (0.61)
		HF	57 (1.71)	38 (1.73)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
	181 to 365 days	VTE	10 (0.30)	< 5	< 5	< 5
		DVT	6 (0.18)	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	14 (0.42)	9 (0.41)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	7 (0.21)	< 5	< 5	< 5
		HF	38 (1.14)	20 (0.91)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 2			N = 594,262	N = 969,262	N = 180,670	N = 302,999
	0 to 30 days	VTE	62 (1.04)	158 (1.63)	5 (0.28)	19 (0.63)
		DVT	18 (0.30)	47 (0.48)	< 5	< 5
		PE	45 (0.76)	120 (1.24)	< 5	15 (0.50)
		ATE	29 (0.49)	75 (0.77)	< 5	< 5
		IS	< 5	12 (0.12)	< 5	< 5
		TIA	11 (0.19)	13 (0.13)	< 5	< 5
		MI	16 (0.27)	52 (0.54)	< 5	< 5
		HF	43 (0.72)	103 (1.06)	< 5	11 (0.36)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	6 (0.06)	< 5	< 5
	31 to 90 days	VTE	21 (0.35)	55 (0.57)	< 5	9 (0.30)
		DVT	7 (0.12)	25 (0.26)	< 5	5 (0.17)
		PE	14 (0.24)	32 (0.33)	< 5	< 5
		ATE	36 (0.61)	57 (0.59)	< 5	5 (0.17)
		IS	5 (0.08)	6 (0.06)	< 5	< 5
		TIA	9 (0.15)	25 (0.26)	< 5	< 5
		MI	22 (0.37)	28 (0.29)	< 5	< 5
		HF	35 (0.59)	68 (0.70)	< 5	7 (0.23)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	10 (0.17)	30 (0.31)	< 5	< 5
		DVT	6 (0.10)	16 (0.17)	< 5	< 5
		PE	5 (0.08)	16 (0.17)	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		ATE	15 (0.25)	28 (0.29)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	5 (0.08)	13 (0.13)	< 5	< 5
		MI	10 (0.17)	15 (0.15)	< 5	< 5
		HF	25 (0.42)	44 (0.45)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	8 (0.13)	5 (0.05)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	7 (0.12)	< 5	< 5	< 5
		ATE	7 (0.12)	11 (0.11)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	7 (0.07)	< 5	< 5
		HF	12 (0.20)	23 (0.24)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 3			**N = 54,102**	**N = 1,473,602**	**N = 36,748**	**N = 423,876**
	0 to 30 days	VTE	< 5	139 (0.94)	< 5	16 (0.38)
		DVT	< 5	41 (0.28)	< 5	< 5
		PE	< 5	101 (0.69)	< 5	12 (0.28)
		ATE	< 5	47 (0.32)	< 5	12 (0.28)
		IS	< 5	5 (0.03)	< 5	< 5
		TIA	< 5	16 (0.11)	< 5	< 5
		MI	< 5	26 (0.18)	< 5	9 (0.21)
		HF	10 (1.85)	28 (0.19)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	6 (0.04)	< 5	< 5
	31 to 90 days	VTE	< 5	44 (0.30)	< 5	7 (0.17)
		DVT	< 5	26 (0.18)	< 5	< 5
		PE	< 5	18 (0.12)	< 5	< 5
		ATE	< 5	31 (0.21)	< 5	7 (0.17)
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	13 (0.09)	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MI	< 5	16 (0.11)	< 5	< 5
		HF	< 5	23 (0.16)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	7 (0.05)	< 5	< 5
	91 to 180 days	VTE	< 5	26 (0.18)	< 5	< 5
		DVT	< 5	15 (0.10)	< 5	< 5
		PE	< 5	14 (0.10)	< 5	< 5
		ATE	< 5	26 (0.18)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	8 (0.05)	< 5	< 5
		MI	< 5	17 (0.12)	< 5	< 5
		HF	< 5	12 (0.08)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	11 (0.07)	< 5	< 5
		DVT	< 5	5 (0.03)	< 5	< 5
		PE	< 5	7 (0.05)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 4			N = 1,335,671	N = 542,670	N = 365,096	N = 147,744
	0 to 30 days	VTE	20 (0.15)	27 (0.50)	< 5	8 (0.54)
		DVT	9 (0.07)	7 (0.13)	< 5	< 5
		PE	11 (0.08)	21 (0.39)	< 5	6 (0.41)
		ATE	< 5	5 (0.09)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	7 (0.05)	14 (0.26)	< 5	< 5
		DVT	< 5	8 (0.15)	< 5	< 5
		PE	< 5	6 (0.11)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	6 (0.04)	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	9 (0.07)	< 5	< 5	< 5
		DVT	7 (0.05)	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S35: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by vaccine (BNT162b2 - ChAdOx1). Follow-up ends at first vaccine dose after index date.

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
Cohort 1			N = 332,790	N = 219,804	N = 32,755	N = 82,406
	0 to 30 days	VTE	31 (0.93)	14 (0.64)	< 5	< 5
		DVT	8 (0.24)	< 5	< 5	< 5
		PE	24 (0.72)	13 (0.59)	< 5	< 5
		ATE	18 (0.54)	10 (0.45)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	14 (0.42)	6 (0.27)	< 5	< 5
		HF	45 (1.35)	28 (1.27)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	15 (0.45)	5 (0.23)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	11 (0.33)	< 5	< 5	< 5
		ATE	8 (0.24)	8 (0.36)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	36 (1.08)	20 (0.91)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	< 5	5 (0.23)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	10 (0.30)	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	32 (0.96)	19 (0.86)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
	181 to 365 days	VTE	7 (0.21)	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	11 (0.33)	8 (0.36)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	6 (0.18)	< 5	< 5	< 5
		HF	35 (1.05)	18 (0.82)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 2			N = 594,262	N = 969,262	N = 180,670	N = 302,999
	0 to 30 days	VTE	14 (0.24)	40 (0.41)	< 5	< 5
		DVT	6 (0.10)	13 (0.13)	< 5	< 5
		PE	8 (0.13)	30 (0.31)	< 5	< 5
		ATE	14 (0.24)	11 (0.11)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	8 (0.13)	9 (0.09)	< 5	< 5
		HF	15 (0.25)	32 (0.33)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	5 (0.08)	8 (0.08)	< 5	< 5
		DVT	< 5	5 (0.05)	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	9 (0.15)	9 (0.09)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	6 (0.06)	< 5	< 5
		HF	17 (0.29)	13 (0.13)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	< 5	12 (0.12)	< 5	< 5
		DVT	< 5	7 (0.07)	< 5	< 5
		PE	< 5	5 (0.05)	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		ATE	5 (0.08)	10 (0.10)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	6 (0.06)	< 5	< 5
		HF	9 (0.15)	18 (0.19)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	7 (0.12)	5 (0.05)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	5 (0.08)	< 5	< 5	< 5
		ATE	6 (0.10)	10 (0.10)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	6 (0.06)	< 5	< 5
		HF	11 (0.19)	20 (0.21)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 3			**N = 54,102**	**N = 1,473,602**	**N = 36,748**	**N = 423,876**
	0 to 30 days	VTE	< 5	23 (0.16)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	19 (0.13)	< 5	< 5
		ATE	< 5	8 (0.05)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	7 (0.05)	< 5	< 5
		HF	7 (1.29)	11 (0.07)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	< 5	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	6 (0.04)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MI	< 5	< 5	< 5	< 5
		HF	< 5	5 (0.03)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	< 5	8 (0.05)	< 5	< 5
		DVT	< 5	6 (0.04)	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	7 (0.05)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	9 (0.06)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	6 (0.04)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 4			N = 1,335,671	N = 542,670	N = 365,096	N = 147,744
	0 to 30 days	VTE	9 (0.07)	8 (0.15)	< 5	6 (0.41)
		DVT	5 (0.04)	< 5	< 5	< 5
		PE	< 5	7 (0.13)	< 5	6 (0.41)
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	< 5	8 (0.15)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	5 (0.09)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	6 (0.04)	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	8 (0.06)	< 5	< 5	< 5
		DVT	7 (0.05)	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Table S36: Number of records (and risk per 10,000 individuals) for post COVID-19 cardiac and thromboembolic complications, across cohorts and databases, stratified by vaccine (BNT162b2 - ChAdOx1). Only first outcome after COVID-19 captured.

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
Cohort 1			N = 332,790	N = 219,804	N = 32,755	N = 82,406
	0 to 30 days	VTE	76 (2.28)	41 (1.87)	< 5	< 5
		DVT	15 (0.45)	12 (0.55)	< 5	< 5
		PE	64 (1.92)	31 (1.41)	< 5	< 5
		ATE	45 (1.35)	25 (1.14)	< 5	6 (0.73)
		IS	5 (0.15)	< 5	< 5	< 5
		TIA	11 (0.33)	7 (0.32)	< 5	< 5
		MI	29 (0.87)	17 (0.77)	< 5	< 5
		HF	126 (3.79)	72 (3.28)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	25 (0.75)	14 (0.64)	< 5	< 5
		DVT	7 (0.21)	8 (0.36)	< 5	< 5
		PE	18 (0.54)	7 (0.32)	< 5	< 5
		ATE	23 (0.69)	20 (0.91)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	10 (0.30)	9 (0.41)	< 5	< 5
		MI	11 (0.33)	9 (0.41)	< 5	< 5
		HF	70 (2.10)	39 (1.77)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	< 5	15 (0.68)	< 5	< 5
		DVT	< 5	5 (0.23)	< 5	< 5
		PE	< 5	10 (0.45)	< 5	< 5
		ATE	18 (0.54)	10 (0.45)	< 5	6 (0.73)
		IS	6 (0.18)	< 5	< 5	< 5
		TIA	6 (0.18)	6 (0.27)	< 5	< 5
		MI	6 (0.18)	< 5	< 5	5 (0.61)
		HF	54 (1.62)	35 (1.59)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
	181 to 365 days	VTE	10 (0.30)	< 5	< 5	< 5
		DVT	6 (0.18)	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	14 (0.42)	9 (0.41)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	7 (0.21)	< 5	< 5	< 5
		HF	35 (1.05)	18 (0.82)	< 5	5 (0.61)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 2			N = 594,262	N = 969,262	N = 180,670	N = 302,999
	0 to 30 days	VTE	62 (1.04)	158 (1.63)	5 (0.28)	19 (0.63)
		DVT	18 (0.30)	47 (0.48)	< 5	< 5
		PE	45 (0.76)	120 (1.24)	< 5	15 (0.50)
		ATE	29 (0.49)	75 (0.77)	< 5	< 5
		IS	< 5	12 (0.12)	< 5	< 5
		TIA	11 (0.19)	13 (0.13)	< 5	< 5
		MI	16 (0.27)	52 (0.54)	< 5	< 5
		HF	43 (0.72)	103 (1.06)	< 5	11 (0.36)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	6 (0.06)	< 5	< 5
	31 to 90 days	VTE	21 (0.35)	54 (0.56)	< 5	9 (0.30)
		DVT	7 (0.12)	25 (0.26)	< 5	5 (0.17)
		PE	14 (0.24)	31 (0.32)	< 5	< 5
		ATE	36 (0.61)	56 (0.58)	< 5	5 (0.17)
		IS	5 (0.08)	6 (0.06)	< 5	< 5
		TIA	9 (0.15)	24 (0.25)	< 5	< 5
		MI	22 (0.37)	28 (0.29)	< 5	< 5
		HF	34 (0.57)	65 (0.67)	< 5	7 (0.23)
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	9 (0.15)	28 (0.29)	< 5	< 5
		DVT	6 (0.10)	15 (0.15)	< 5	< 5
		PE	< 5	15 (0.15)	< 5	< 5

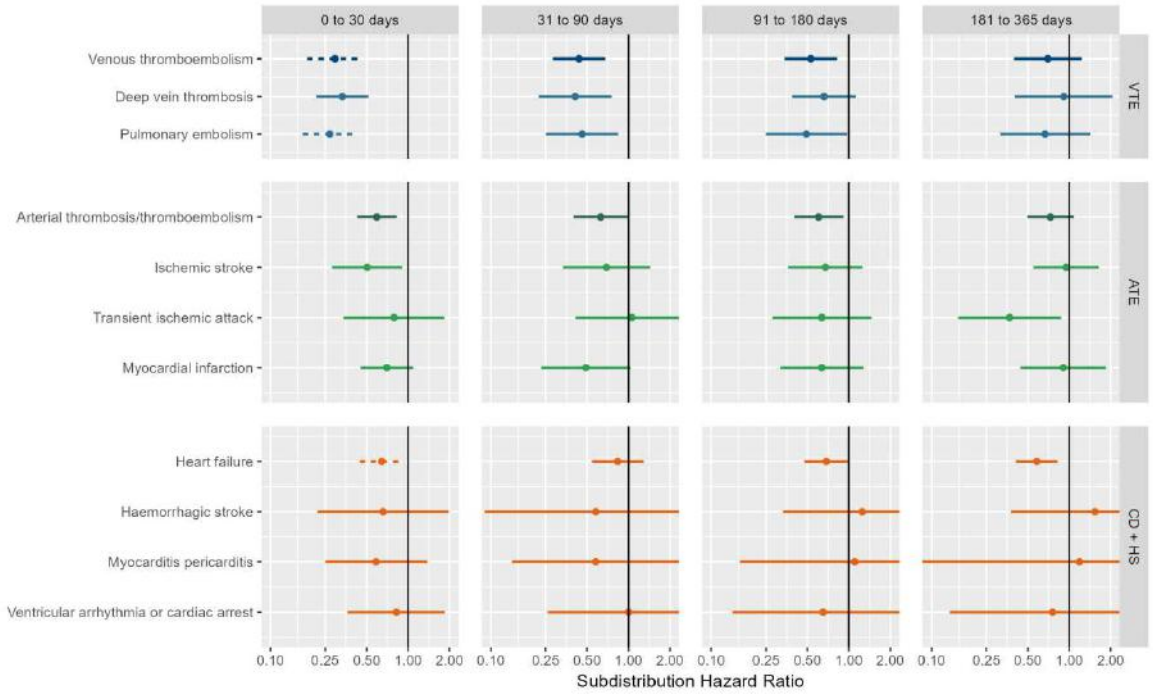
Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		ATE	15 (0.25)	28 (0.29)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	5 (0.08)	13 (0.13)	< 5	< 5
		MI	10 (0.17)	15 (0.15)	< 5	< 5
		HF	24 (0.40)	41 (0.42)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	8 (0.13)	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	7 (0.12)	< 5	< 5	< 5
		ATE	7 (0.12)	10 (0.10)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	6 (0.06)	< 5	< 5
		HF	12 (0.20)	22 (0.23)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 3			**N = 54,102**	**N = 1,473,602**	**N = 36,748**	**N = 423,876**
	0 to 30 days	VTE	< 5	139 (0.94)	< 5	16 (0.38)
		DVT	< 5	41 (0.28)	< 5	< 5
		PE	< 5	101 (0.69)	< 5	12 (0.28)
		ATE	< 5	47 (0.32)	< 5	12 (0.28)
		IS	< 5	5 (0.03)	< 5	< 5
		TIA	< 5	16 (0.11)	< 5	< 5
		MI	< 5	26 (0.18)	< 5	9 (0.21)
		HF	10 (1.85)	28 (0.19)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	6 (0.04)	< 5	< 5
	31 to 90 days	VTE	< 5	44 (0.30)	< 5	6 (0.14)
		DVT	< 5	26 (0.18)	< 5	< 5
		PE	< 5	18 (0.12)	< 5	< 5
		ATE	< 5	31 (0.21)	< 5	7 (0.17)
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	13 (0.09)	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MI	< 5	16 (0.11)	< 5	< 5
		HF	< 5	23 (0.16)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	7 (0.05)	< 5	< 5
	91 to 180 days	VTE	< 5	25 (0.17)	< 5	< 5
		DVT	< 5	15 (0.10)	< 5	< 5
		PE	< 5	13 (0.09)	< 5	< 5
		ATE	< 5	26 (0.18)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	8 (0.05)	< 5	< 5
		MI	< 5	17 (0.12)	< 5	< 5
		HF	< 5	10 (0.07)	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	9 (0.06)	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	6 (0.04)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
Cohort 4			N = 1,335,671	N = 542,670	N = 365,096	N = 147,744
	0 to 30 days	VTE	20 (0.15)	27 (0.50)	< 5	8 (0.54)
		DVT	9 (0.07)	7 (0.13)	< 5	< 5
		PE	11 (0.08)	21 (0.39)	< 5	6 (0.41)
		ATE	< 5	5 (0.09)	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5

Cohort	Time window	Outcome	AURUM		GOLD	
			BNT162b2	ChAdOx1	BNT162b2	ChAdOx1
		MP	< 5	< 5	< 5	< 5
	31 to 90 days	VTE	7 (0.05)	14 (0.26)	< 5	< 5
		DVT	< 5	8 (0.15)	< 5	< 5
		PE	< 5	6 (0.11)	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	6 (0.04)	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	91 to 180 days	VTE	9 (0.07)	< 5	< 5	< 5
		DVT	7 (0.05)	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5
	181 to 365 days	VTE	< 5	< 5	< 5	< 5
		DVT	< 5	< 5	< 5	< 5
		PE	< 5	< 5	< 5	< 5
		ATE	< 5	< 5	< 5	< 5
		IS	< 5	< 5	< 5	< 5
		TIA	< 5	< 5	< 5	< 5
		MI	< 5	< 5	< 5	< 5
		HF	< 5	< 5	< 5	< 5
		HS	< 5	< 5	< 5	< 5
		MP	< 5	< 5	< 5	< 5

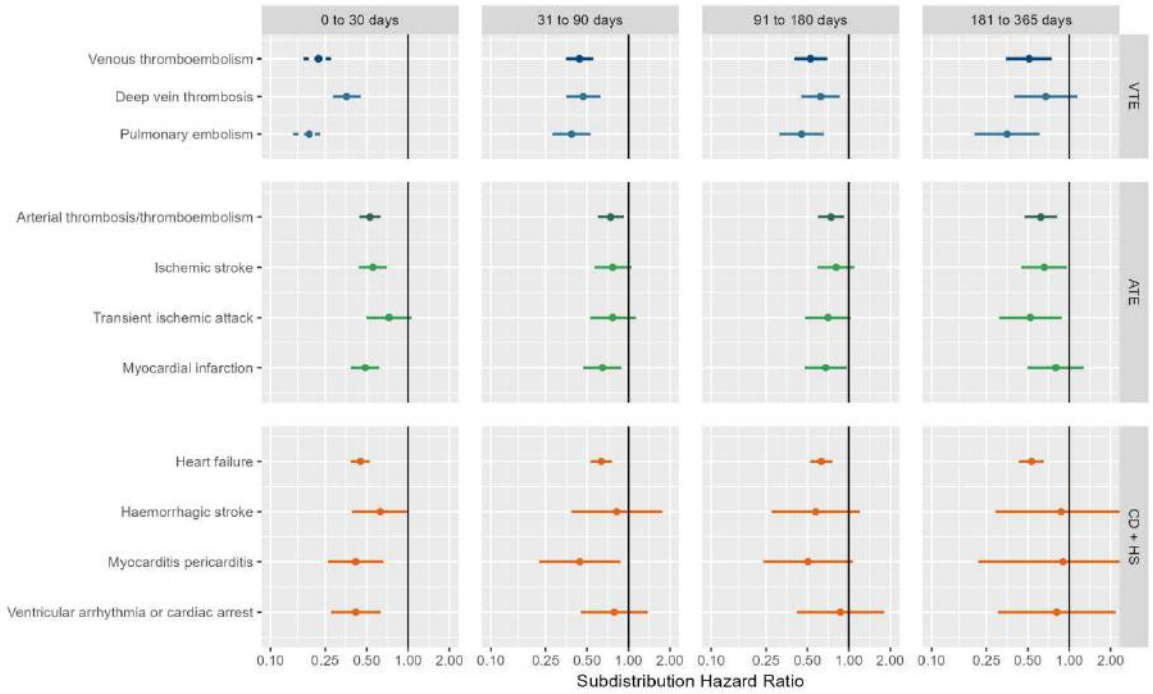
VTE = Venous thromboembolism (DVT + PE), DVT = Deep vein thrombosis, PE = Pulmonary embolism, ATE = Arterial thrombosis/thromboembolism (IS + TIA + MI), IS = Ischemic stroke, TIA = Transient ischemic attack, MI = Myocardial infarction, HF = Heart failure, HS = Hemorrhagic stroke, MP = Myocarditis or Pericarditis

Figure S6: Forest plots for vaccine effect (any COVID-19 vaccine), meta-analysis across cohorts and databases. Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.



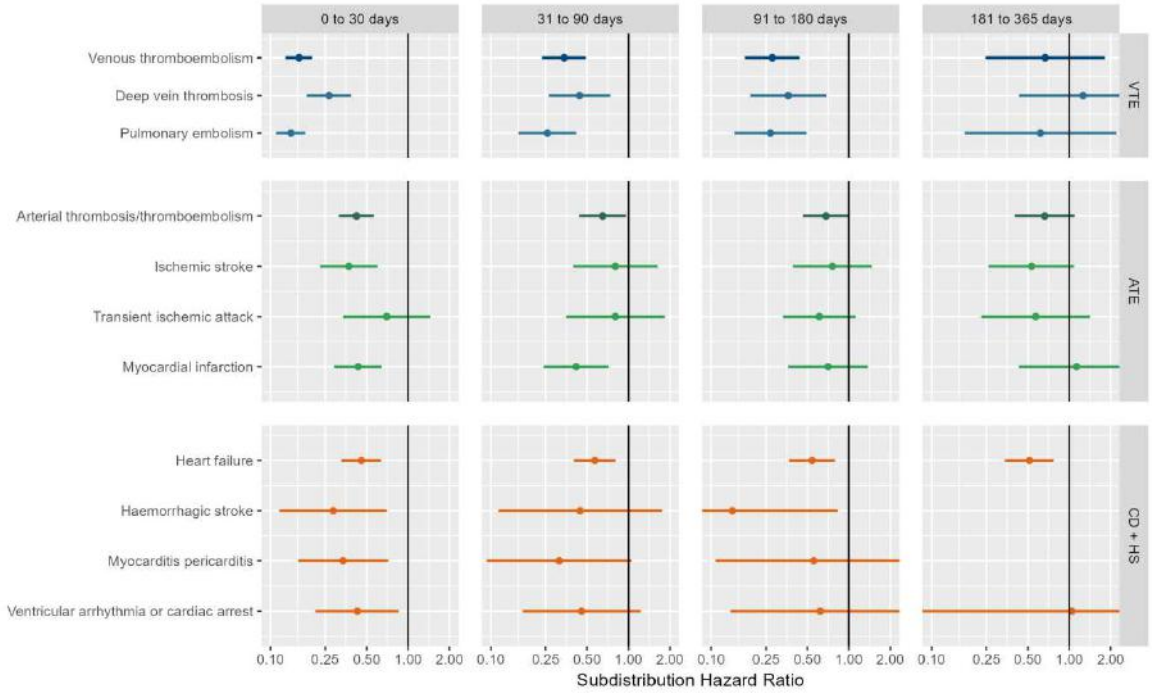
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S7: Forest plots for vaccine effect (any COVID-19 vaccine), meta-analysis across cohorts and databases. Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.



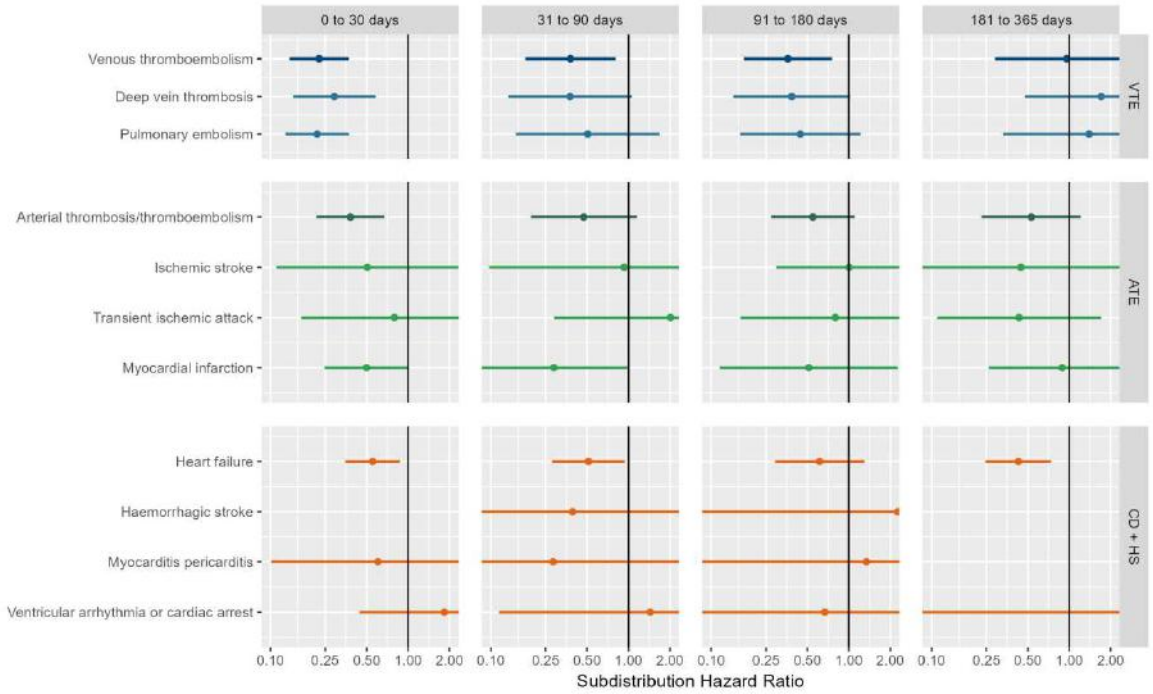
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S8: Forest plots for vaccine effect (ChAdOx1 vaccine), meta-analysis across cohorts and databases. Dashed line represents a level of hetereogeneity I2 > 0.4.



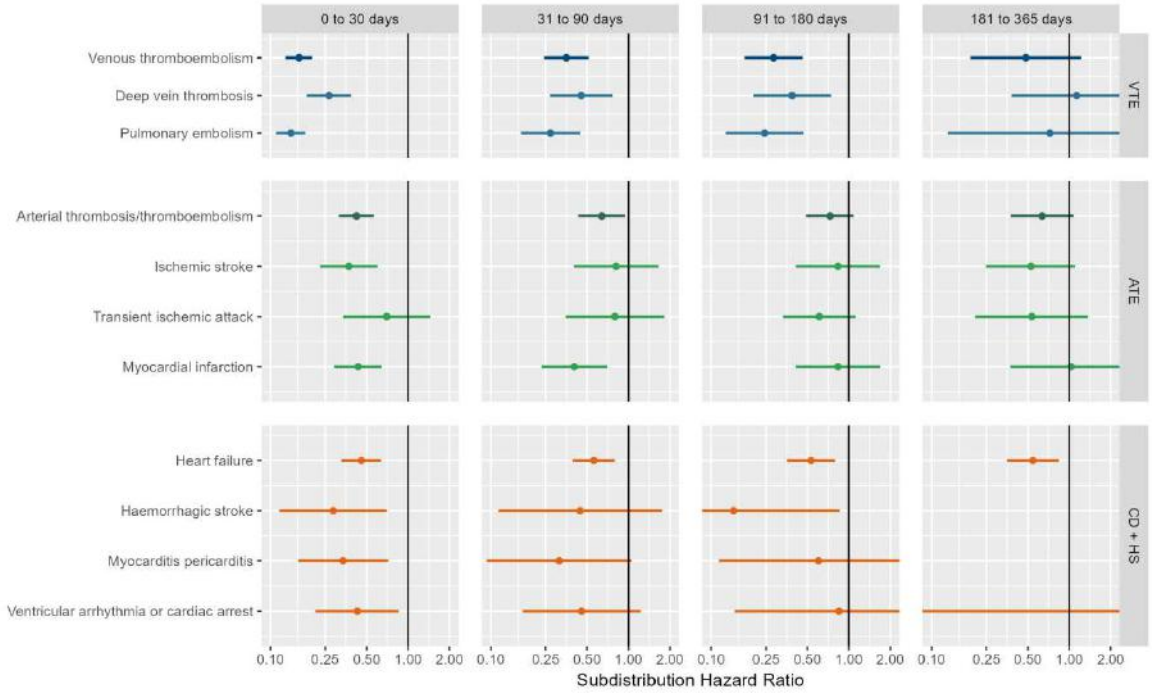
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S9: Forest plots for vaccine effect (ChAdOx1 vaccine), meta-analysis across cohorts and databases. Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.



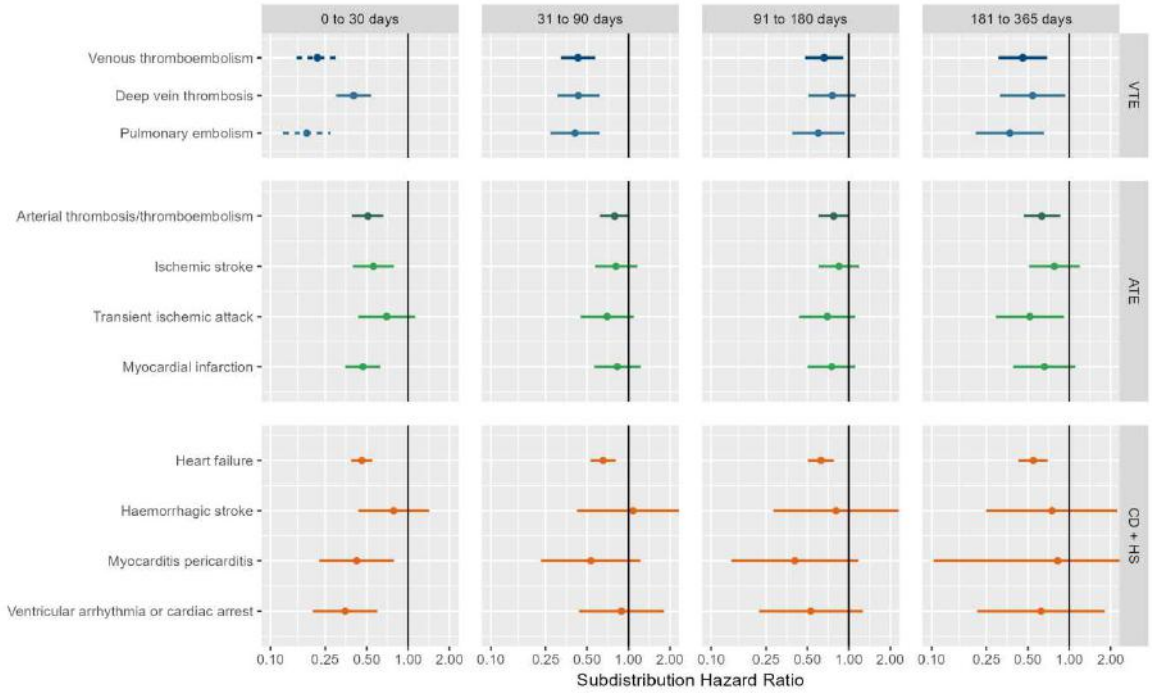
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S10: Forest plots for vaccine effect (ChAdOx1 vaccine), meta-analysis across cohorts and databases. Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.



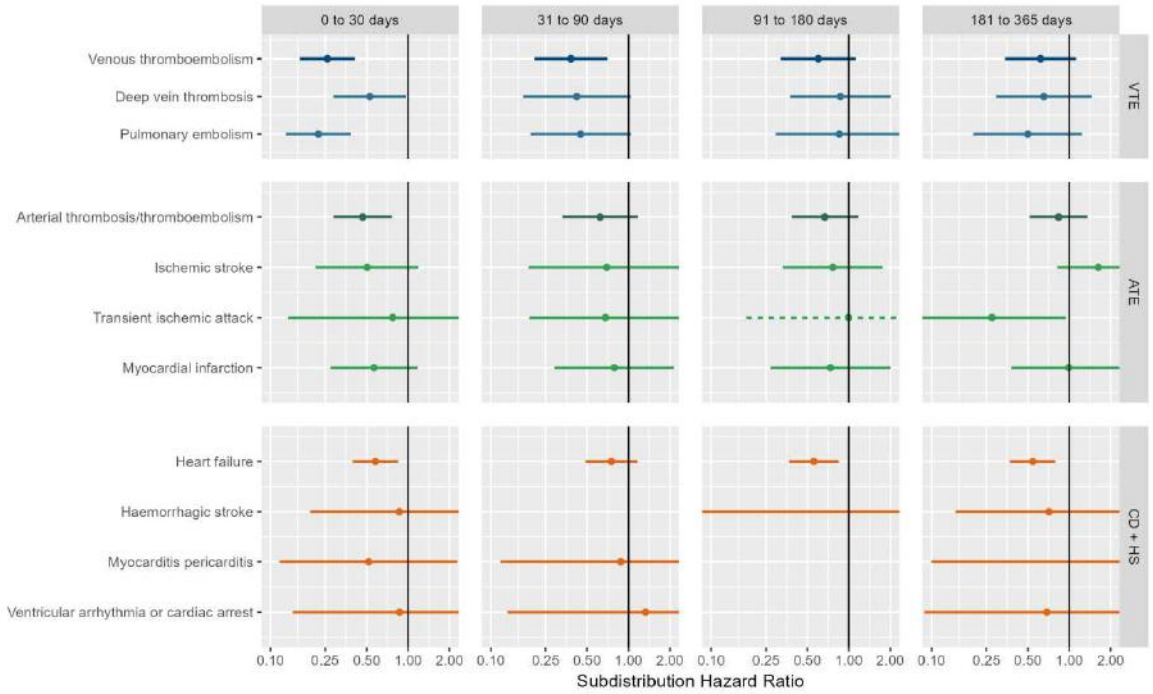
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S11: Forest plots for vaccine effect (BNT162b2 vaccine), meta-analysis across cohorts and databases. Dashed line represents a level of hetereogeneity I2 > 0.4.



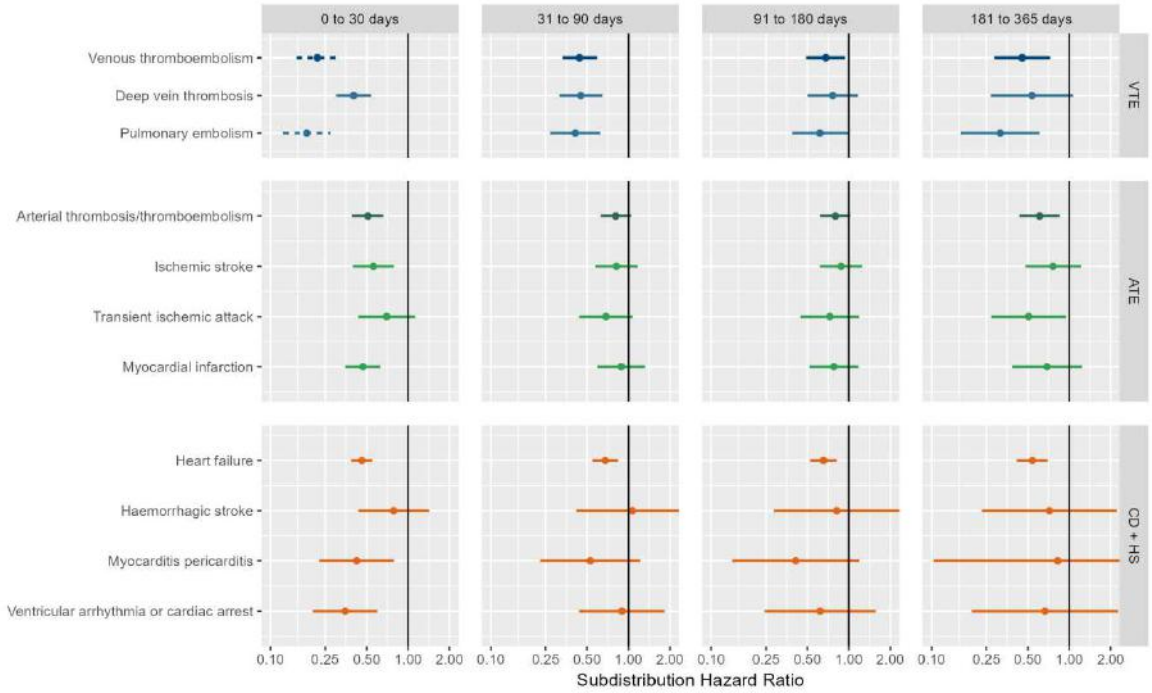
VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S12: Forest plots for vaccine effect (BNT162b2 vaccine), meta-analysis across cohorts and databases. Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.



VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S13: Forest plots for vaccine effect (BNT162b2 vaccine), meta-analysis across cohorts and databases. Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.



VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S14: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

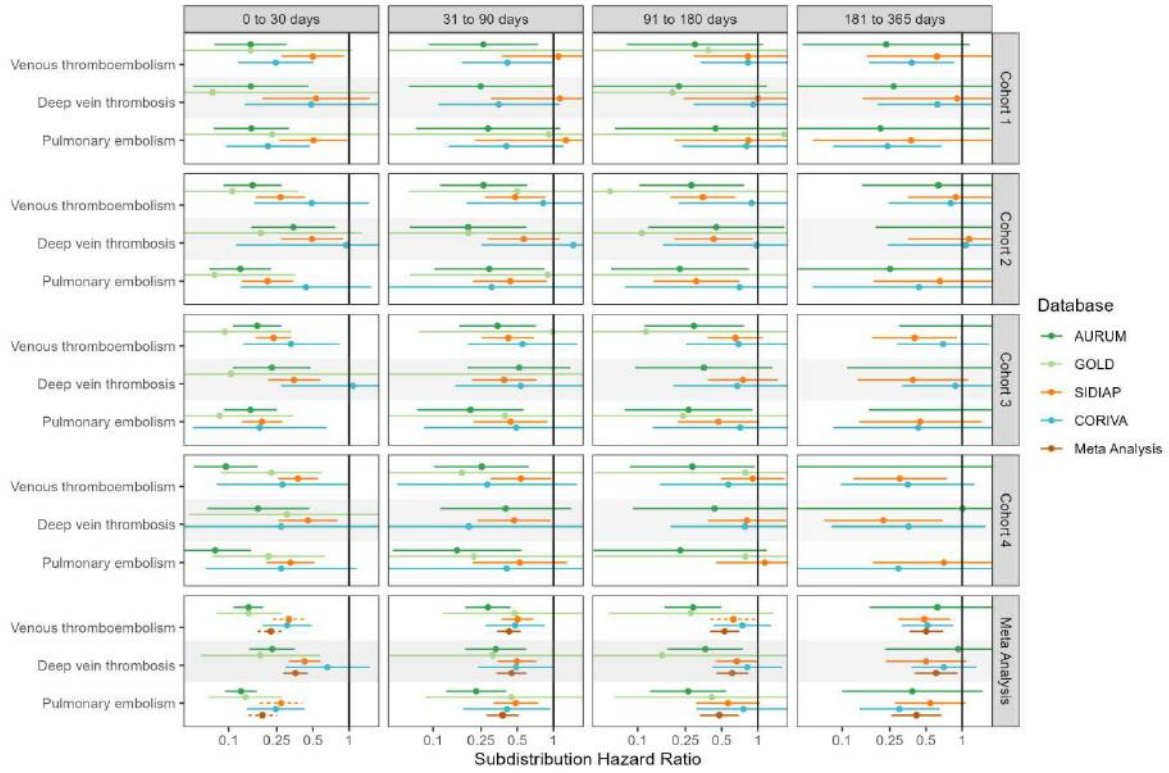


Figure S15: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

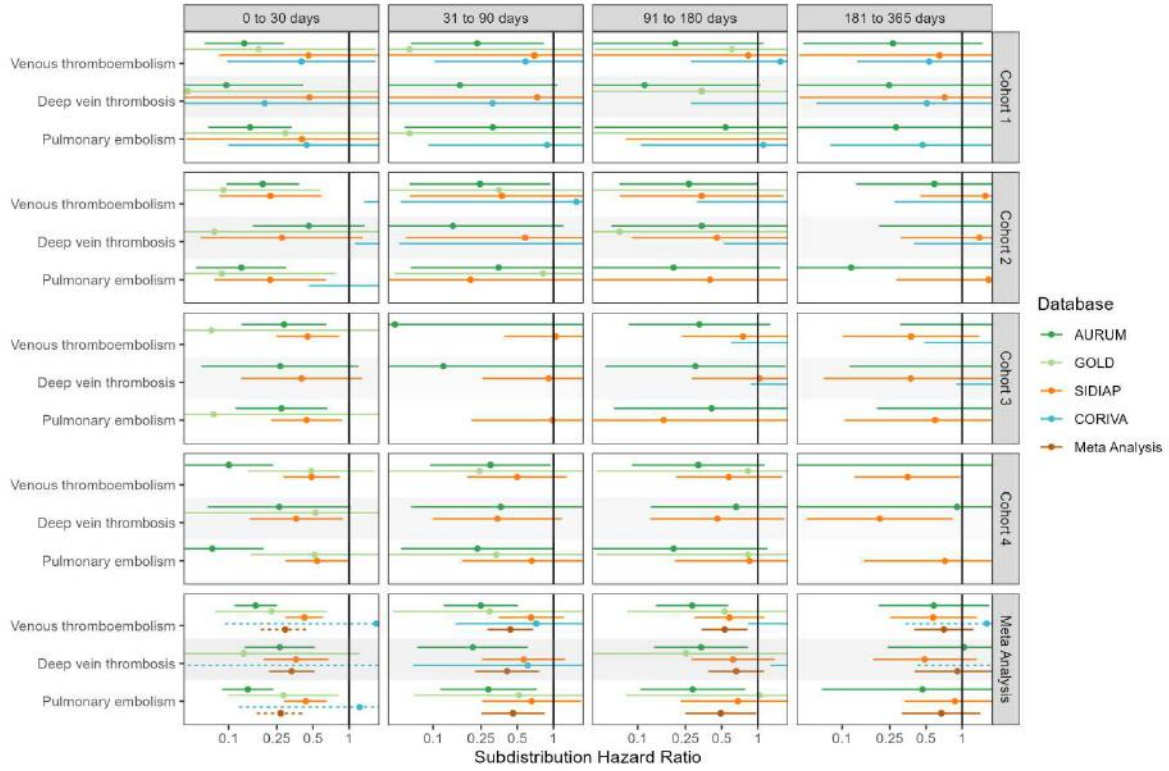


Figure S16: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

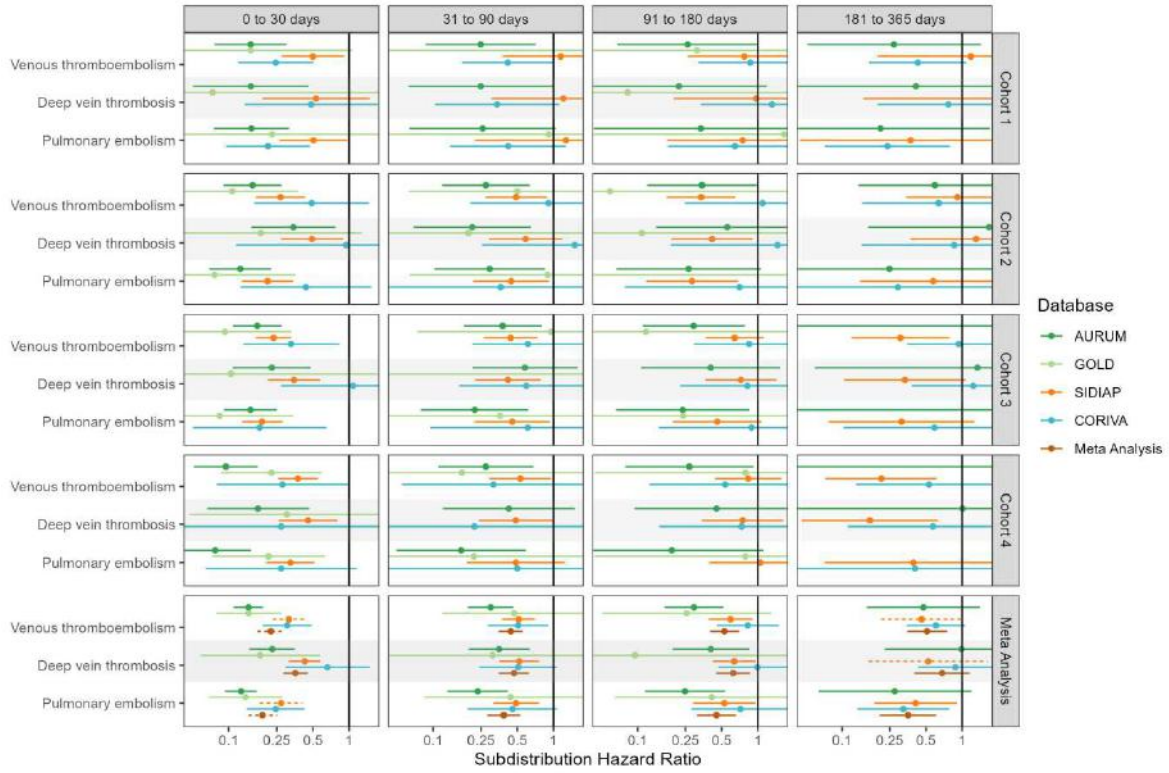


Figure S17: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

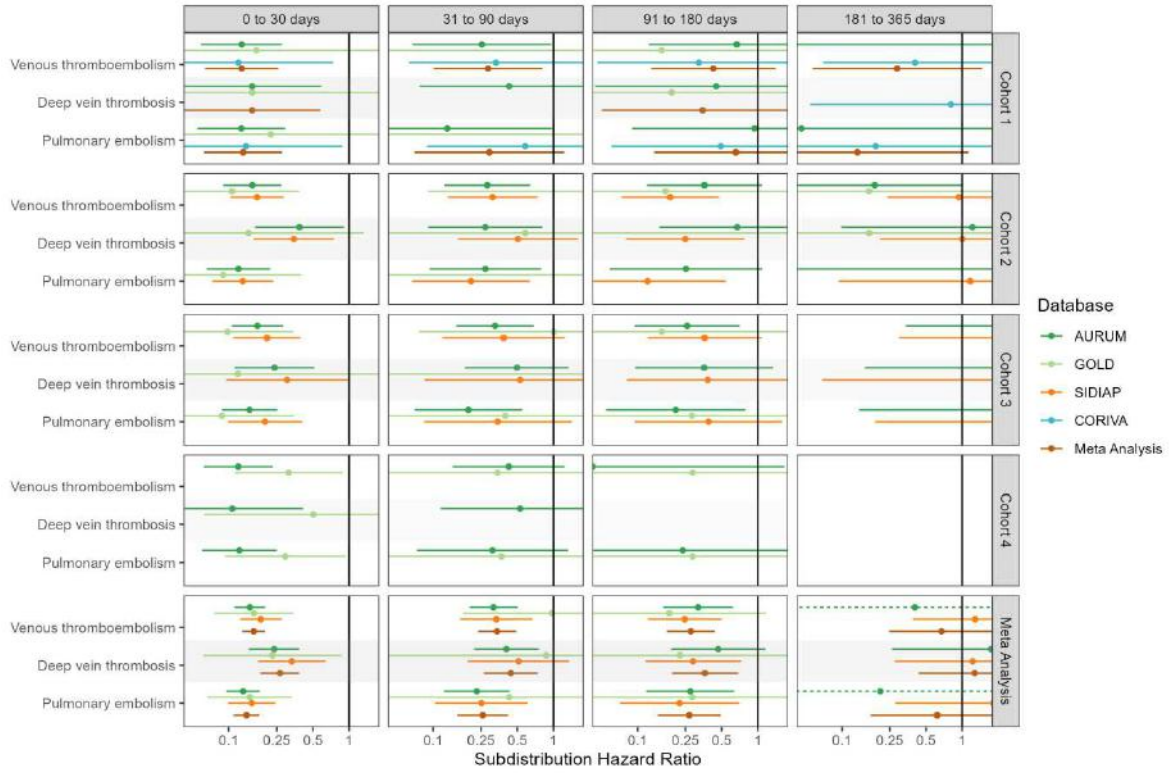


Figure S18: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

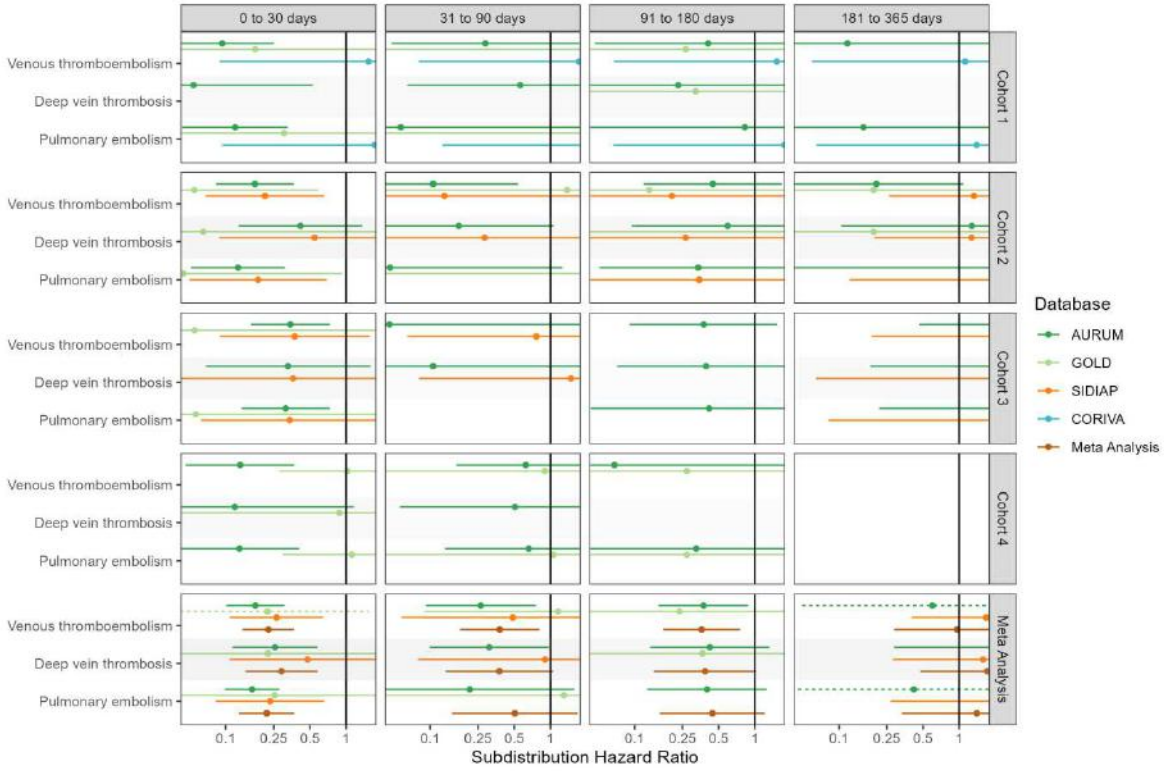


Figure S19: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

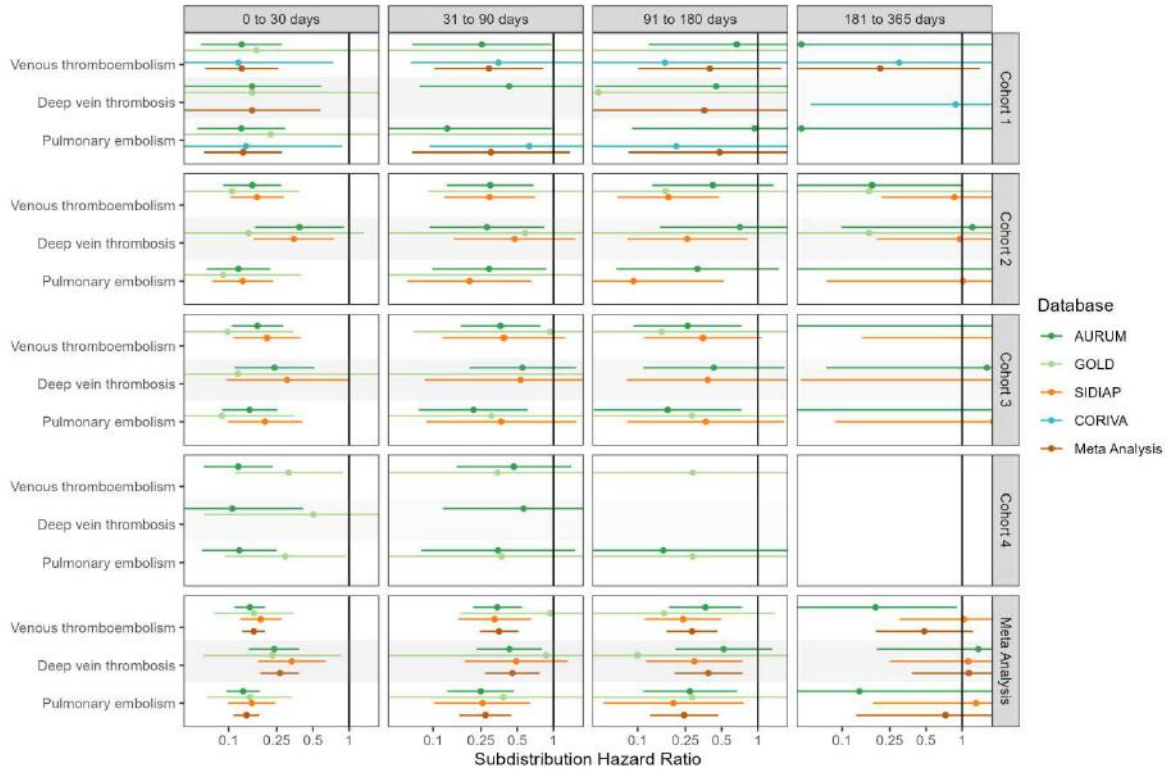


Figure S20: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

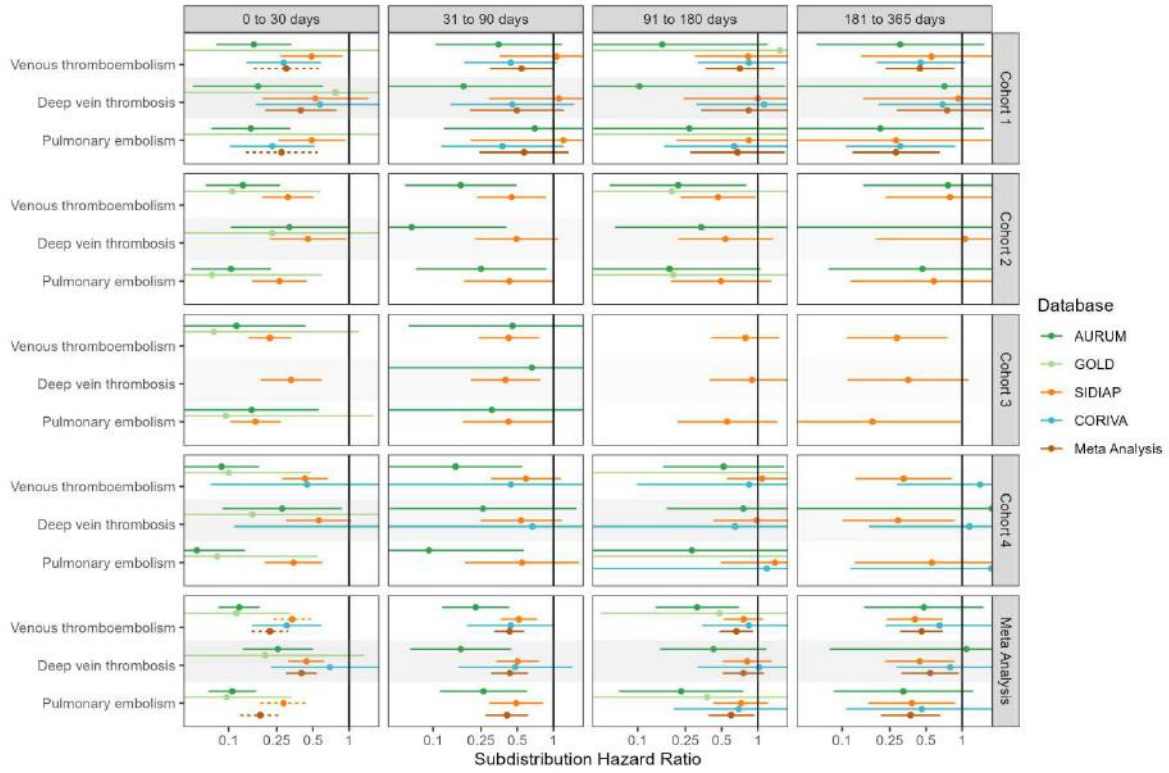


Figure S21: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

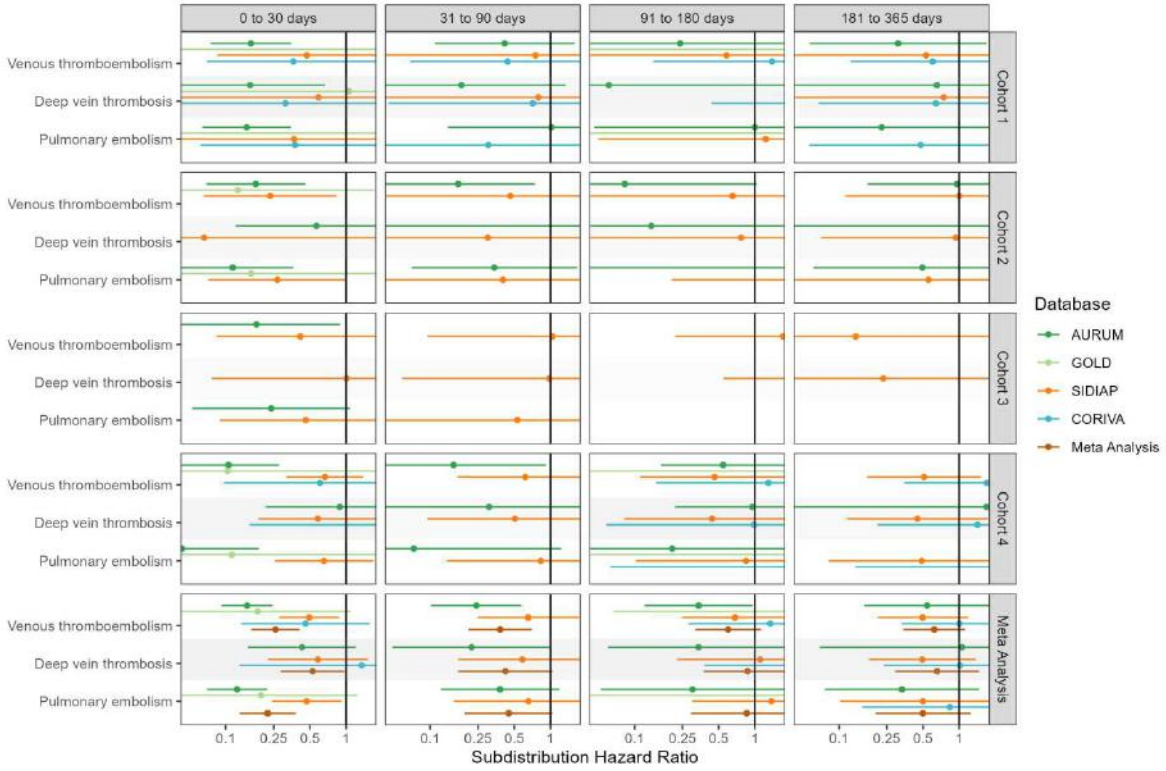


Figure S22: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

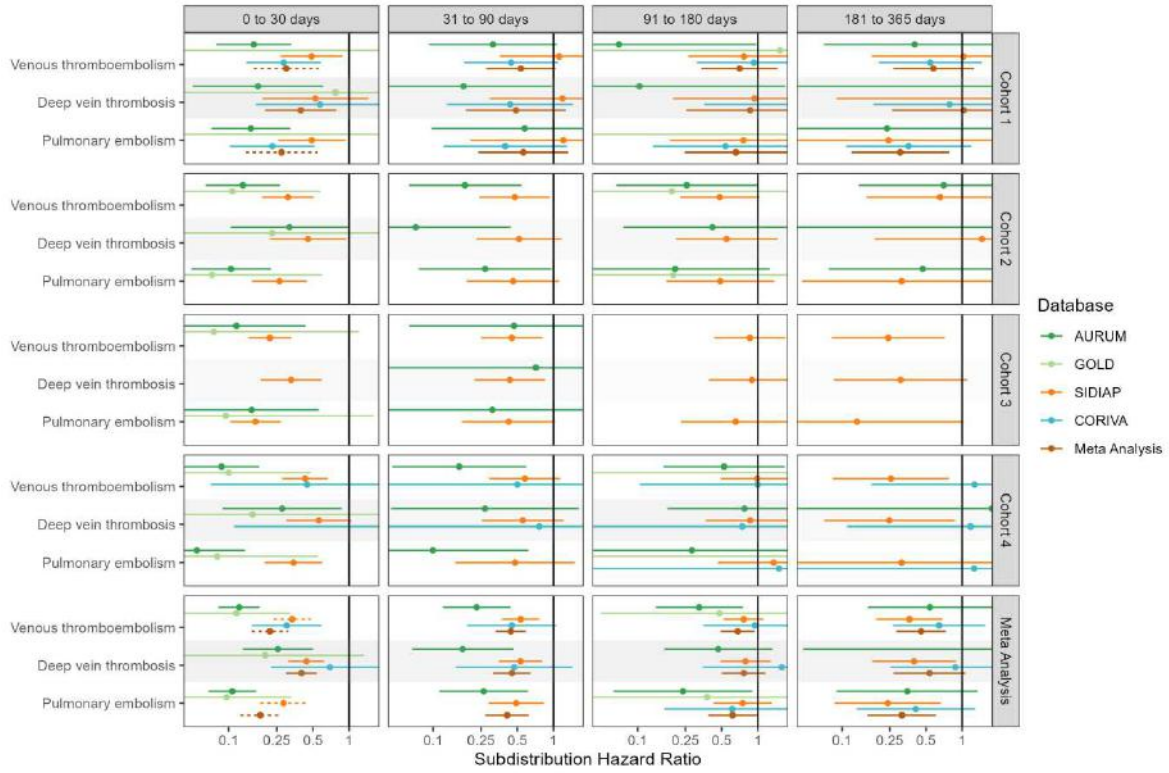


Figure S23: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

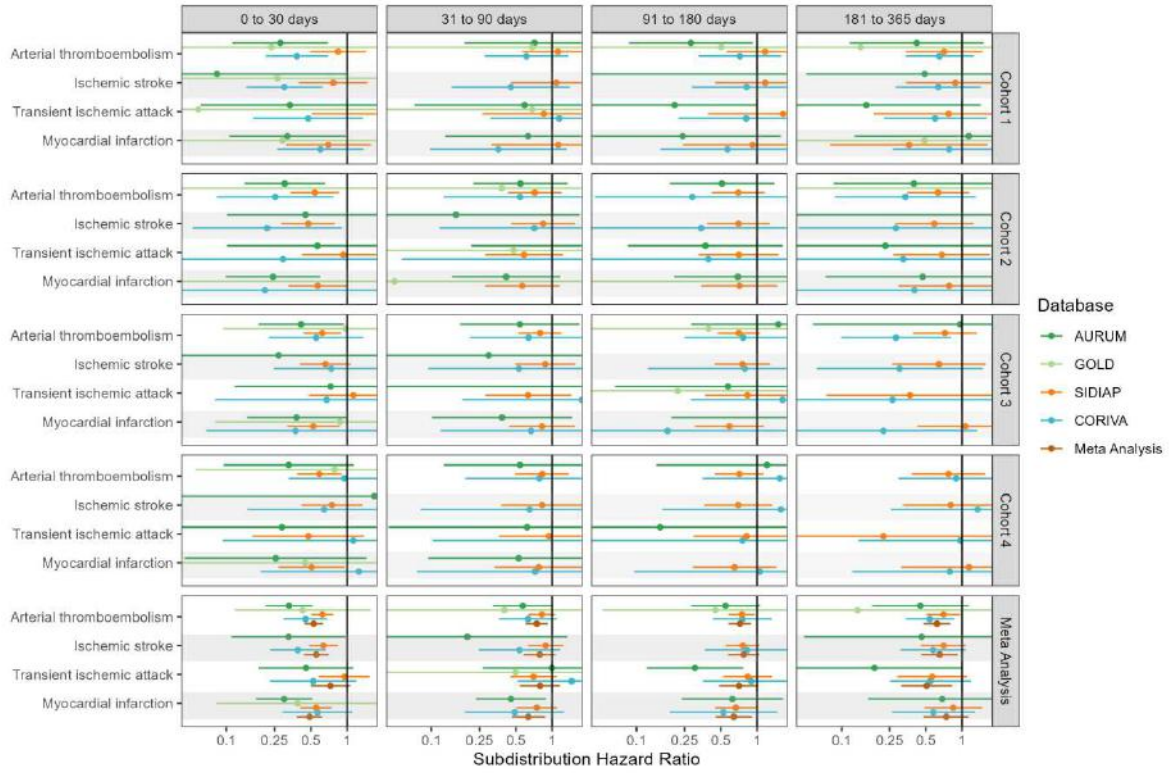


Figure S24: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

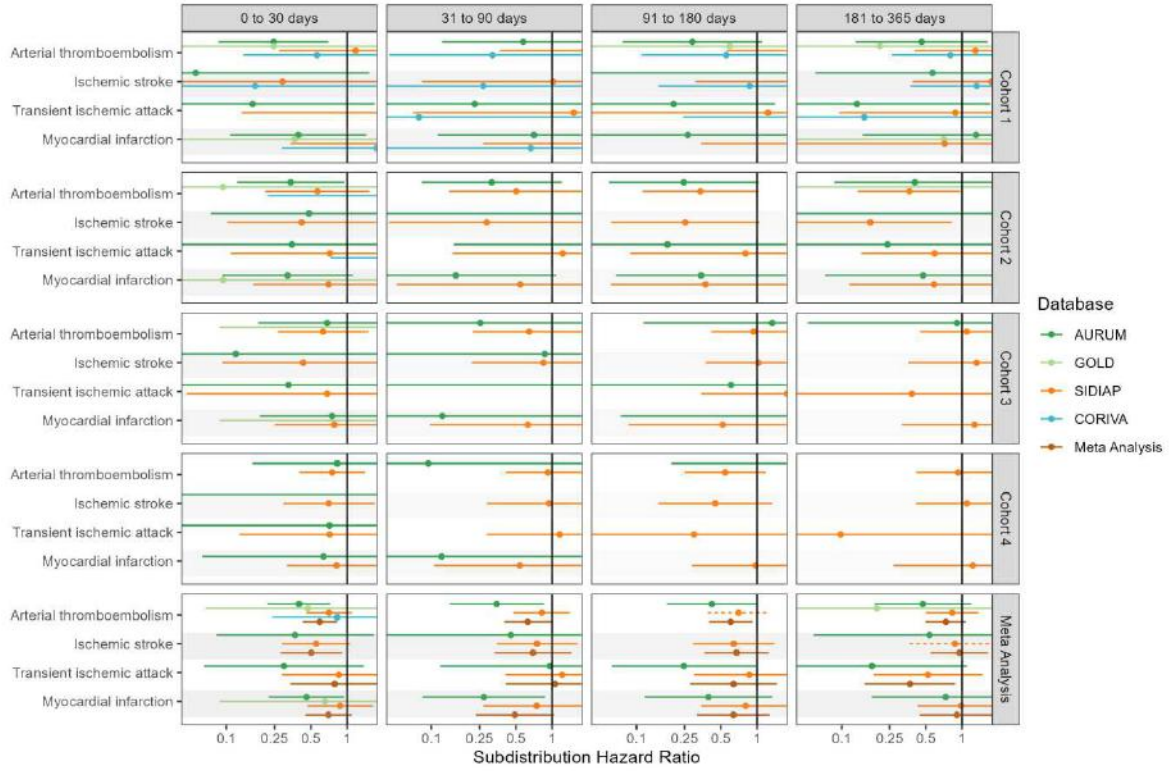


Figure S25: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

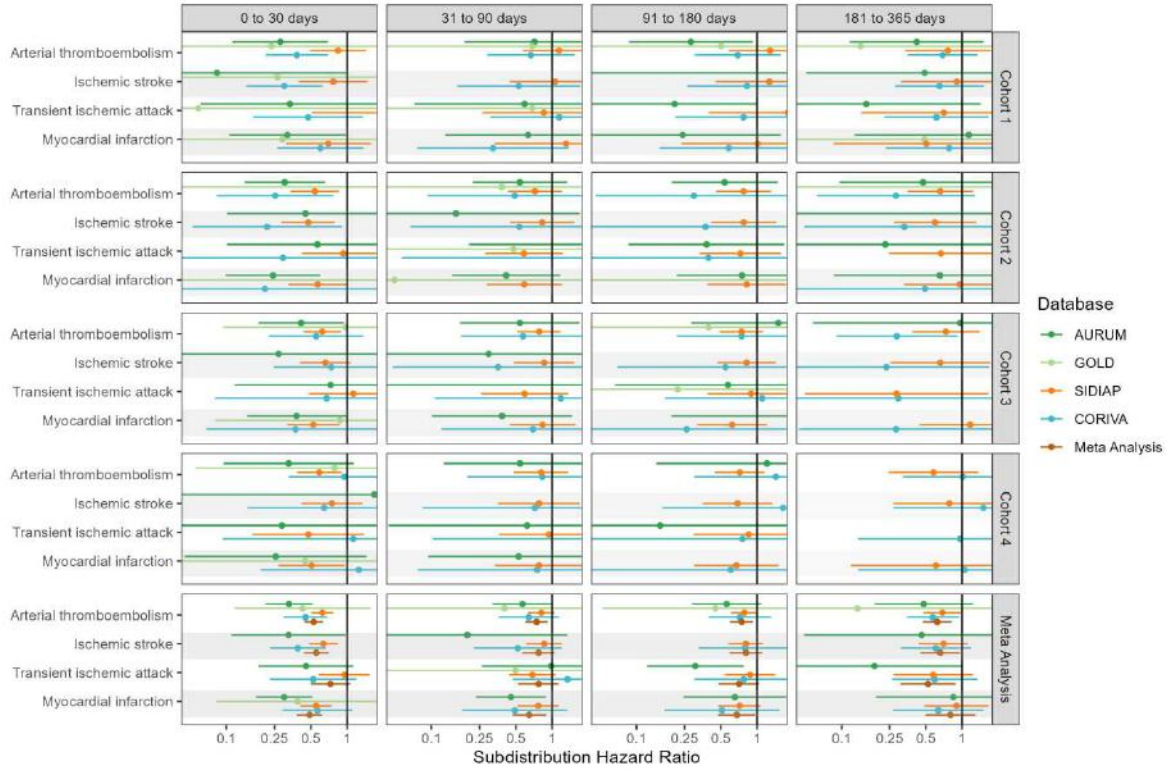


Figure S26: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

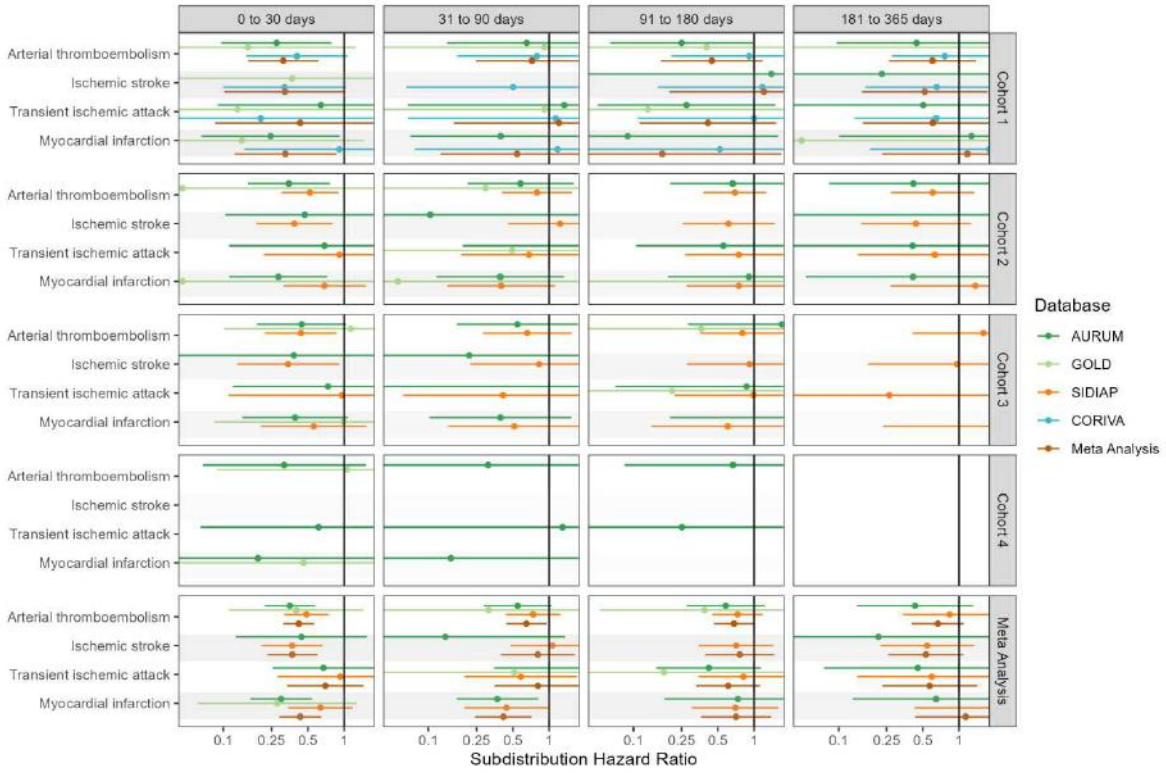


Figure S27: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

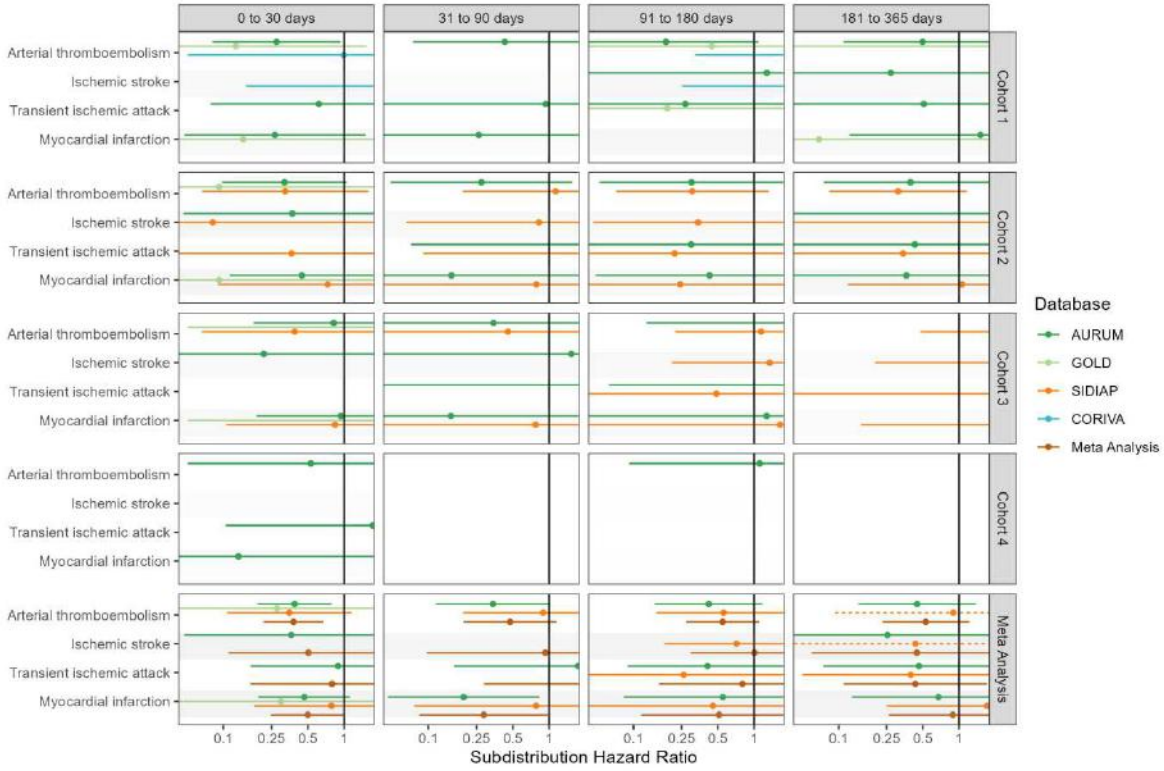


Figure S28: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

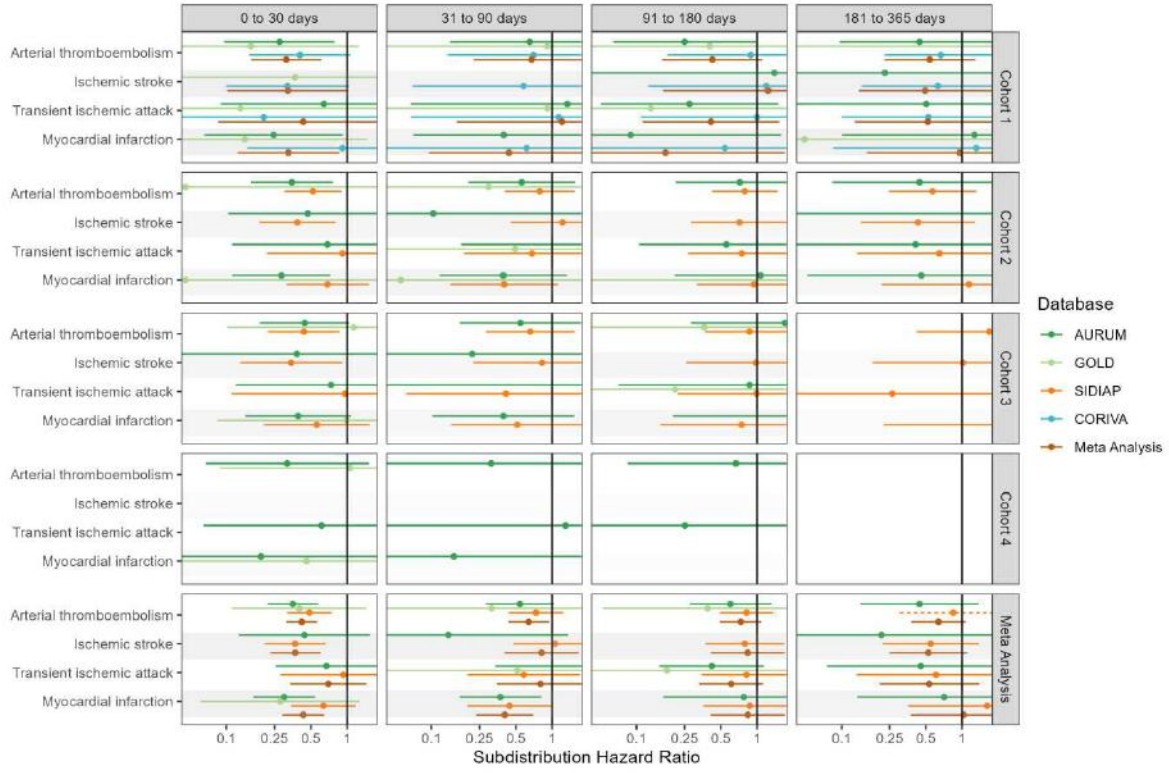


Figure S29: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

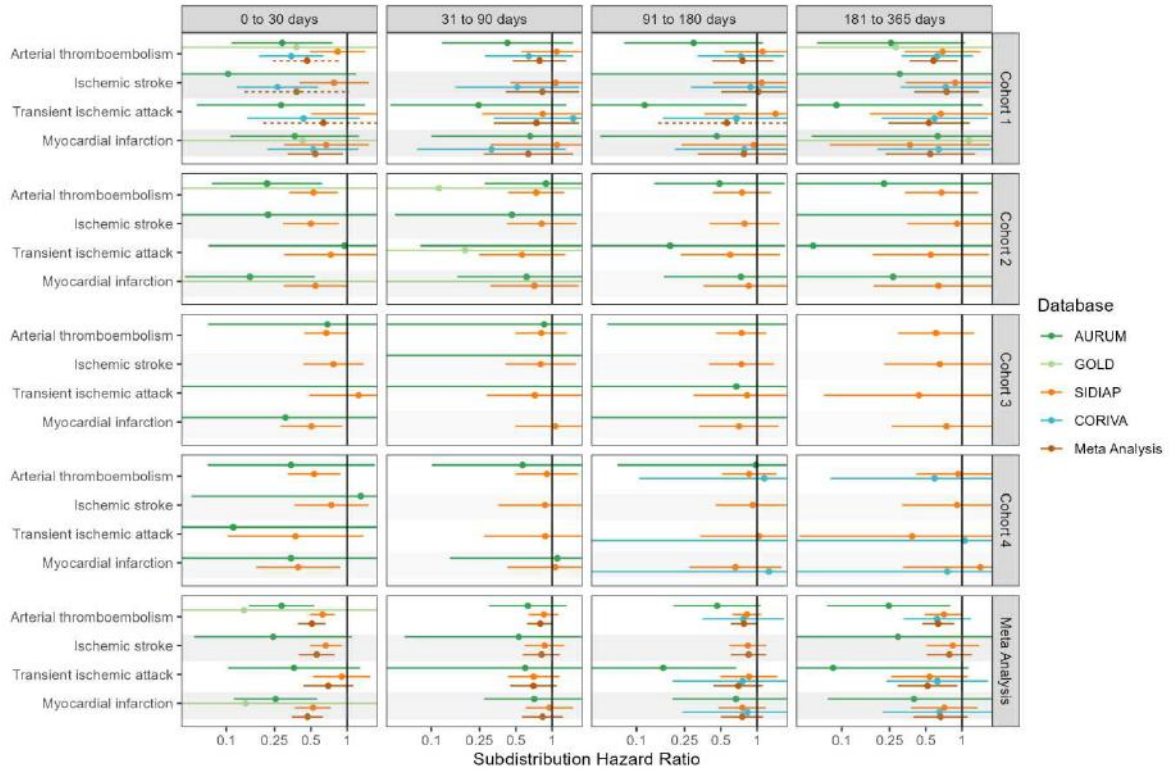


Figure S30: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

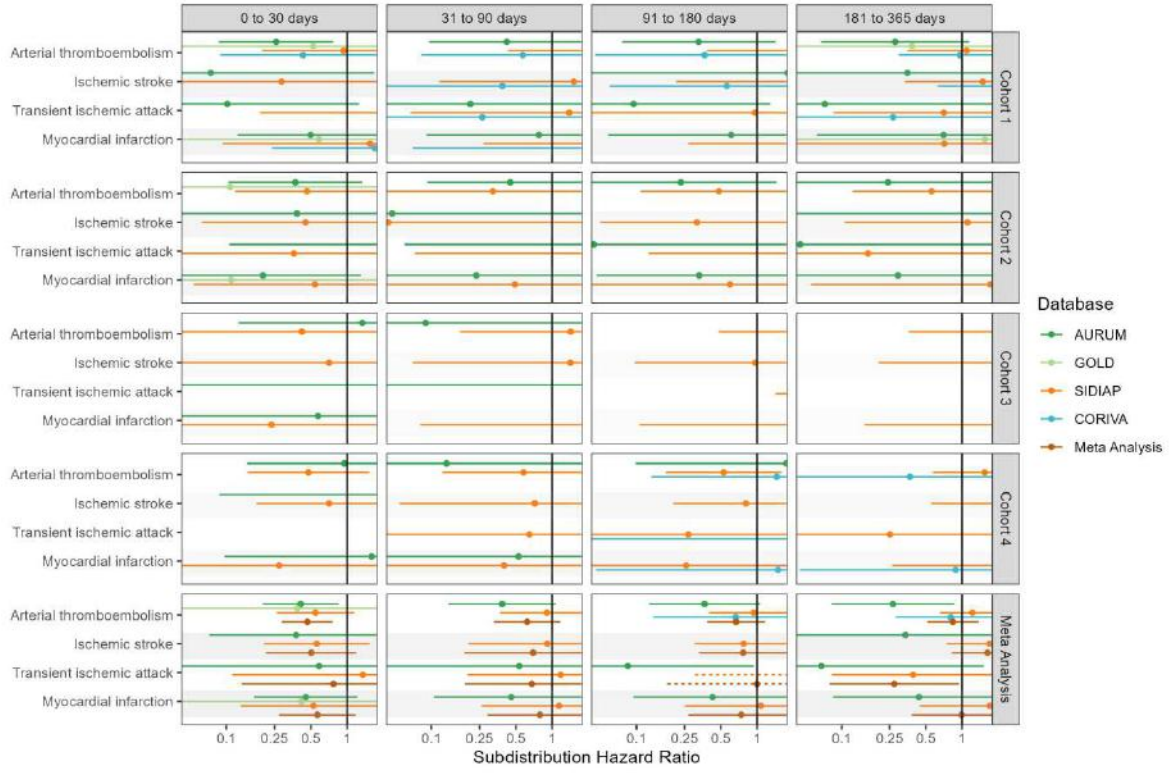


Figure S31: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

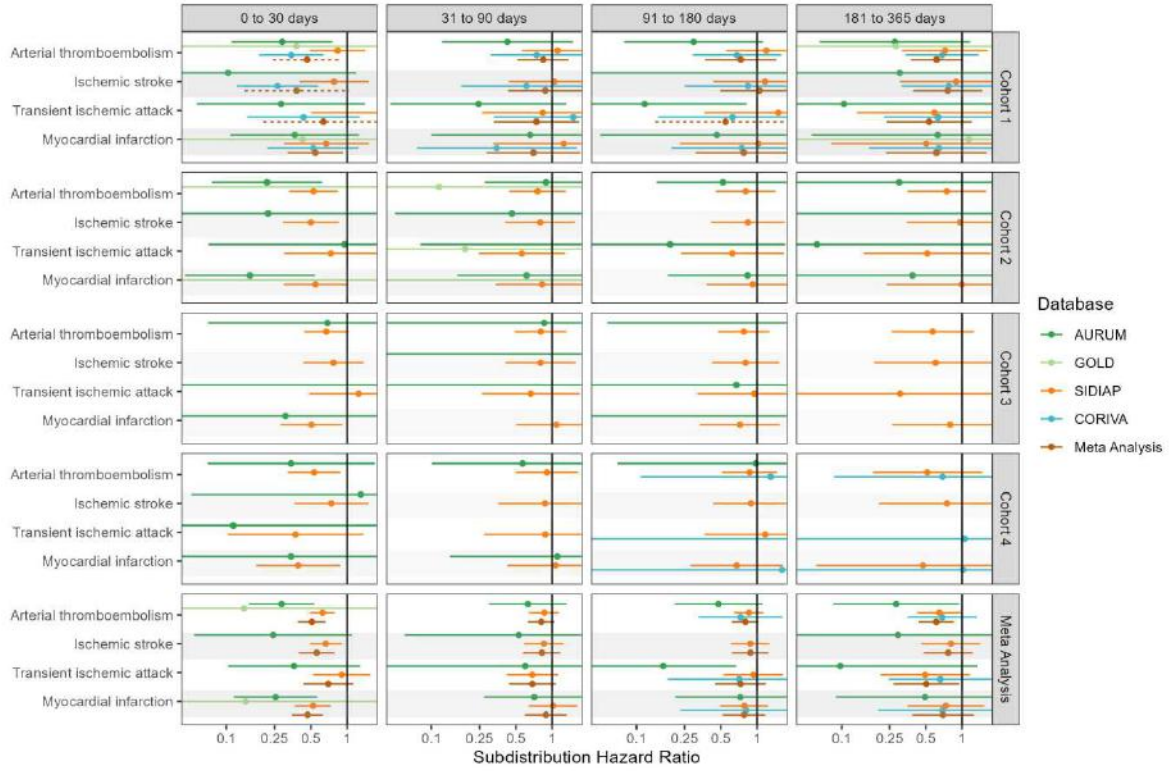


Figure S32: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

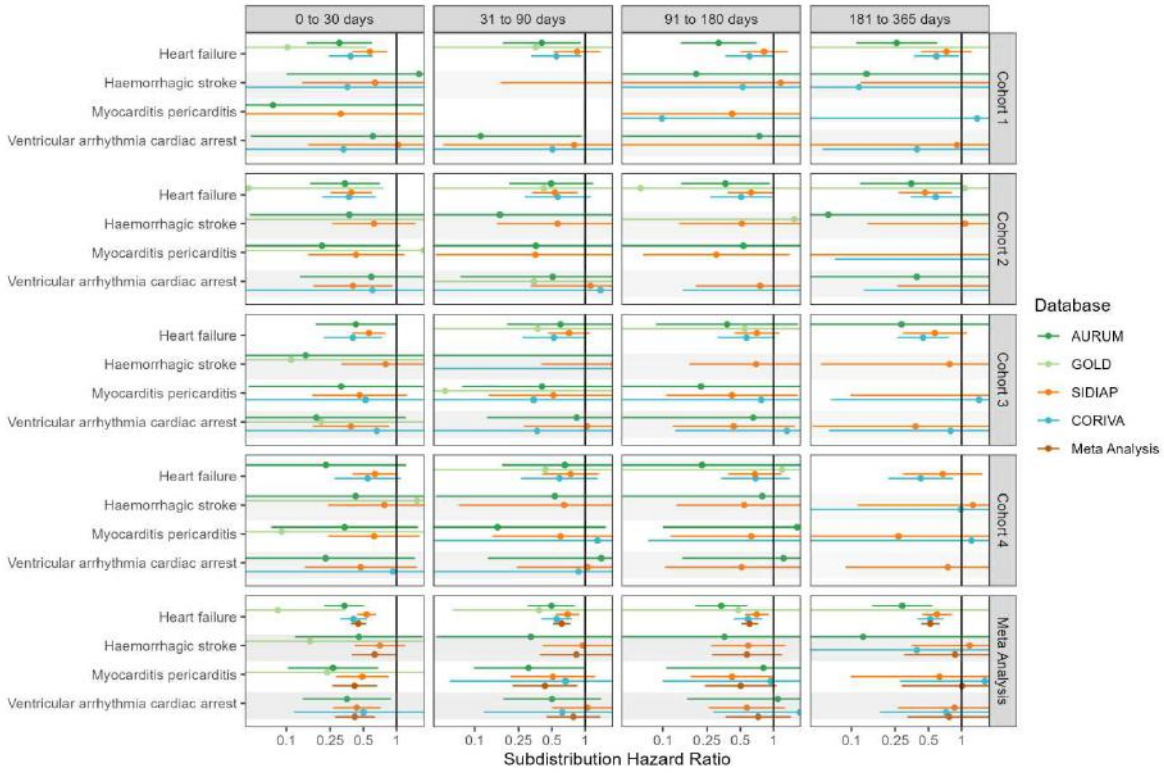


Figure S33: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

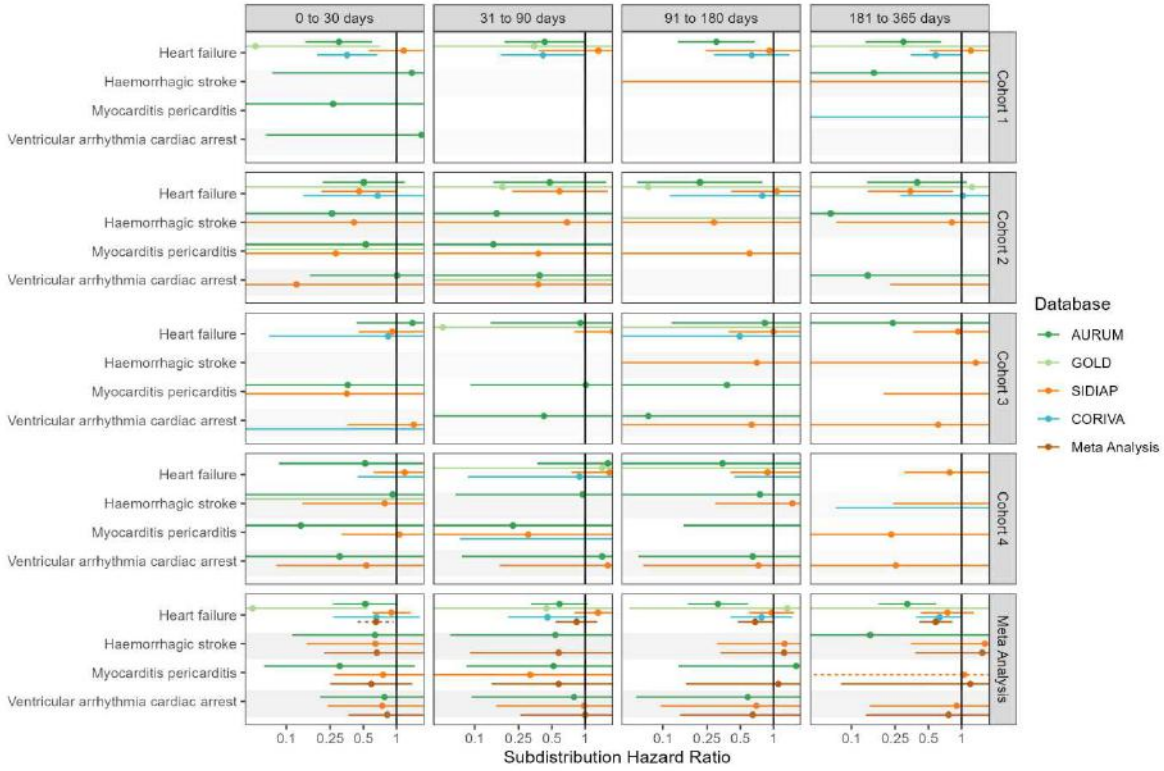


Figure S34: Forest plots for vaccine effect (any COVID-19 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

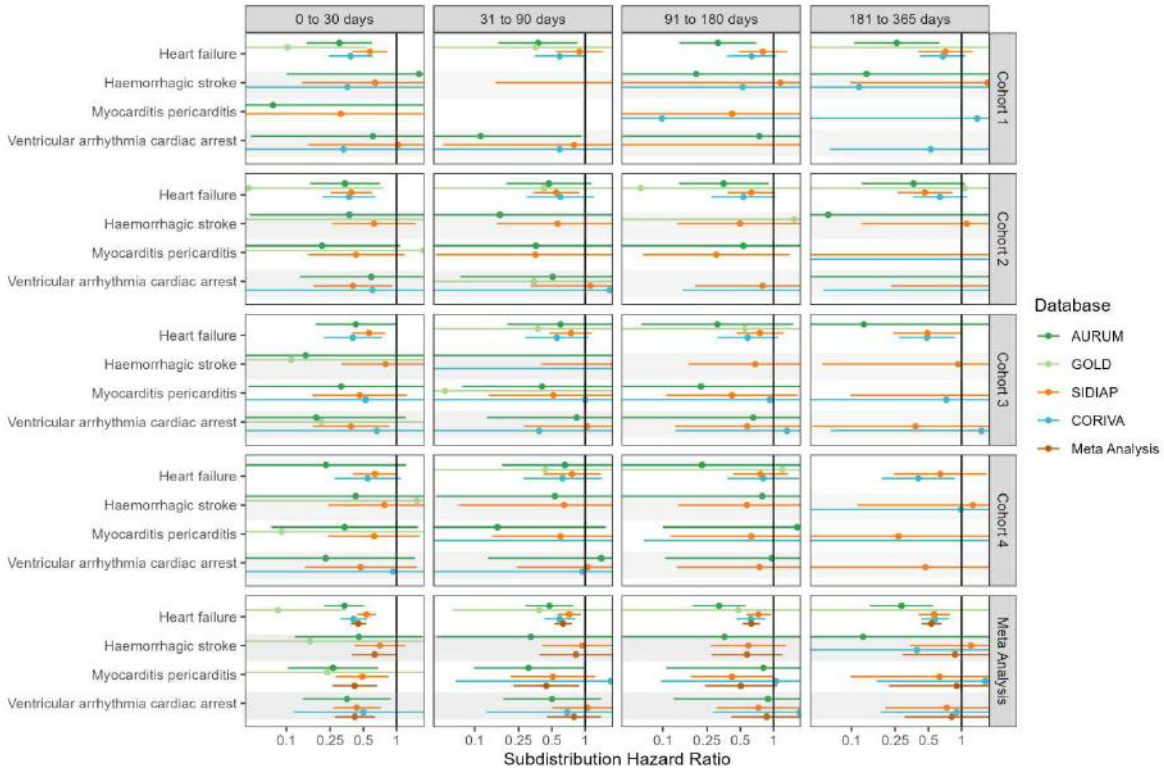


Figure S35: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

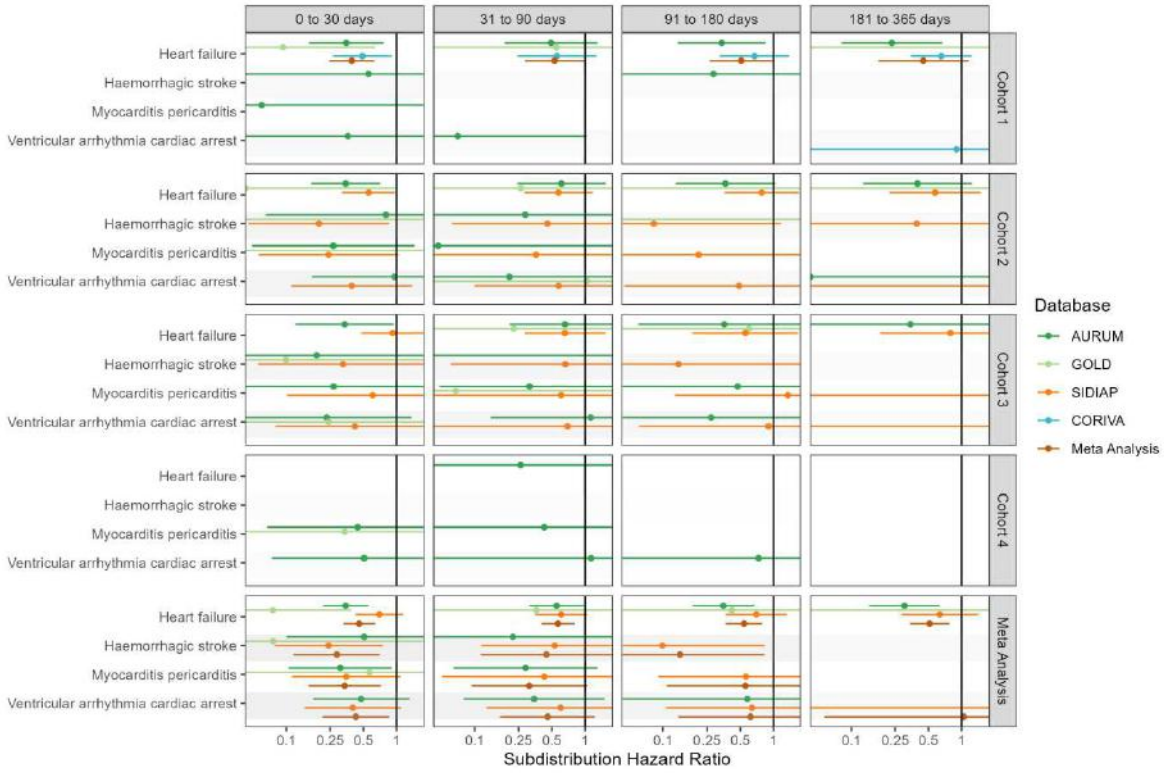


Figure S36: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

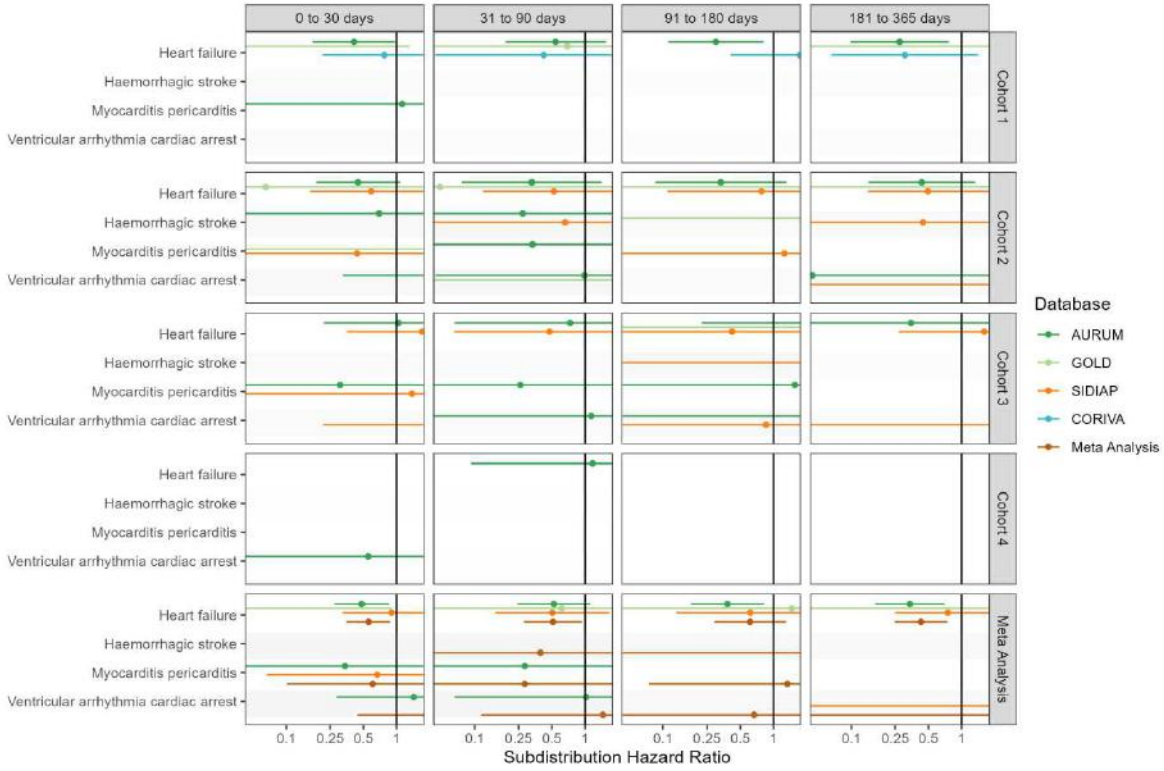


Figure S37: Forest plots for vaccine effect (ChAdOx1 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

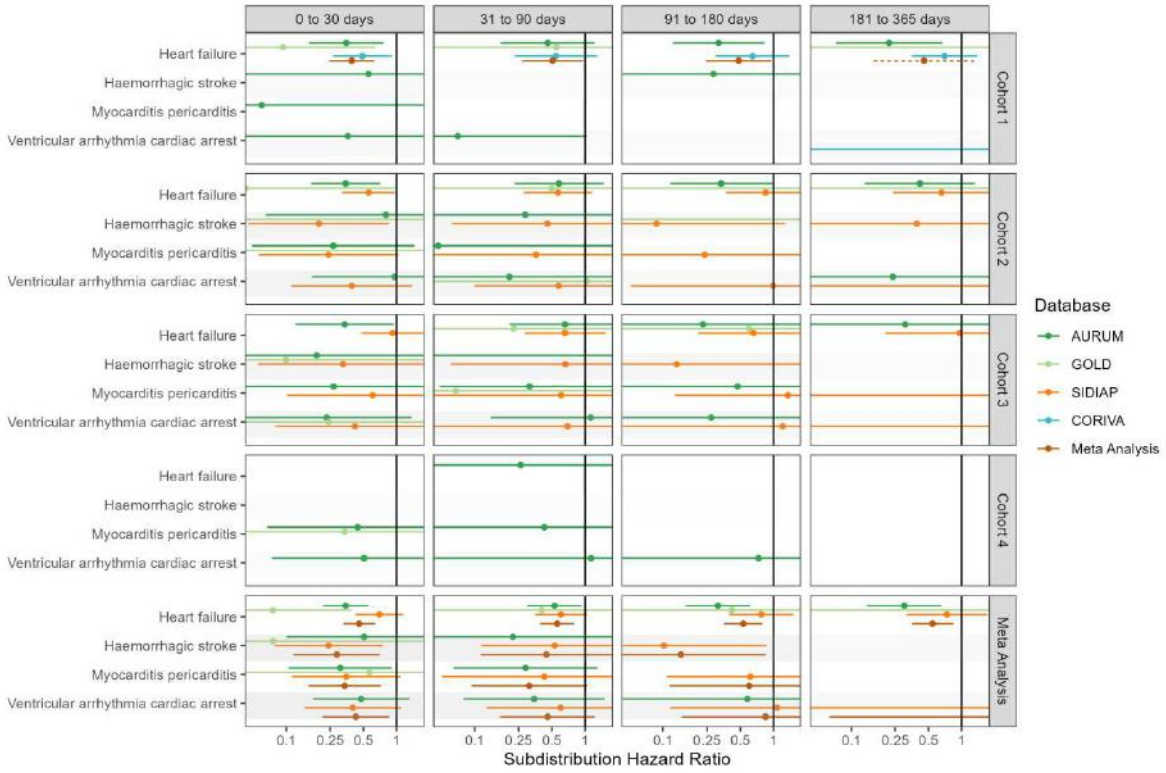


Figure S38: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

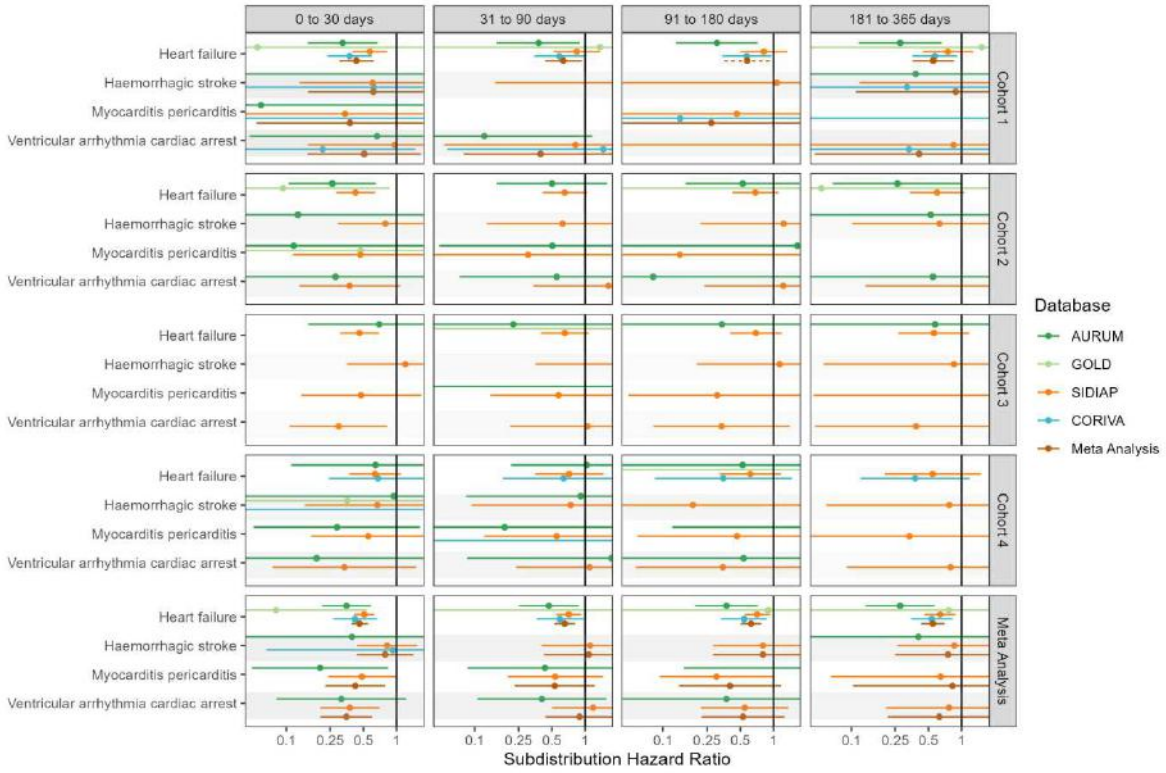


Figure S39: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

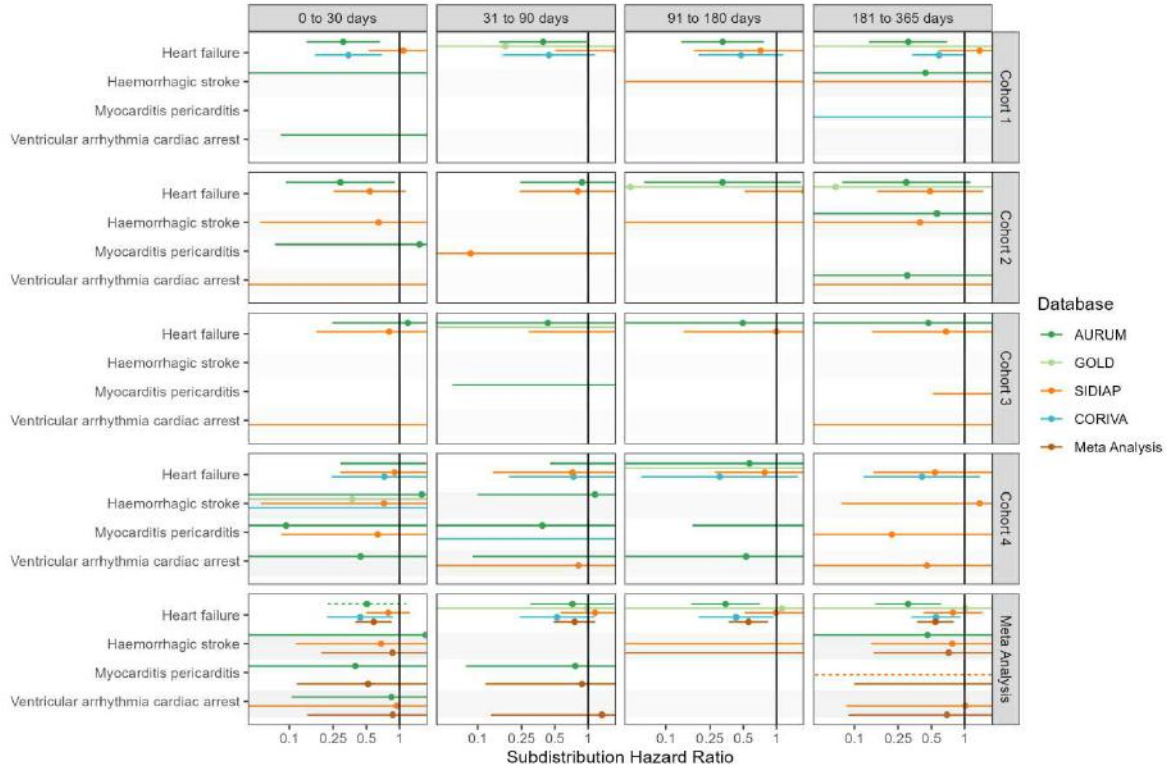


Figure S40: Forest plots for vaccine effect (BNT162b2 vaccine) on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

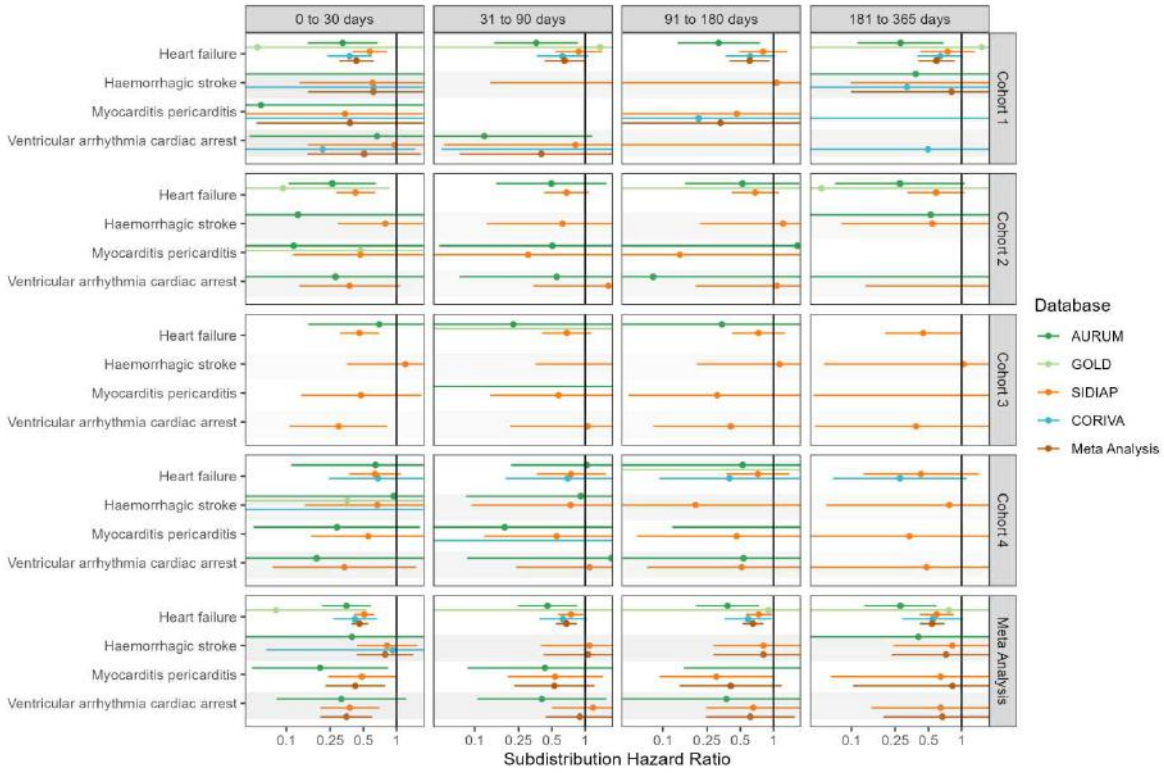
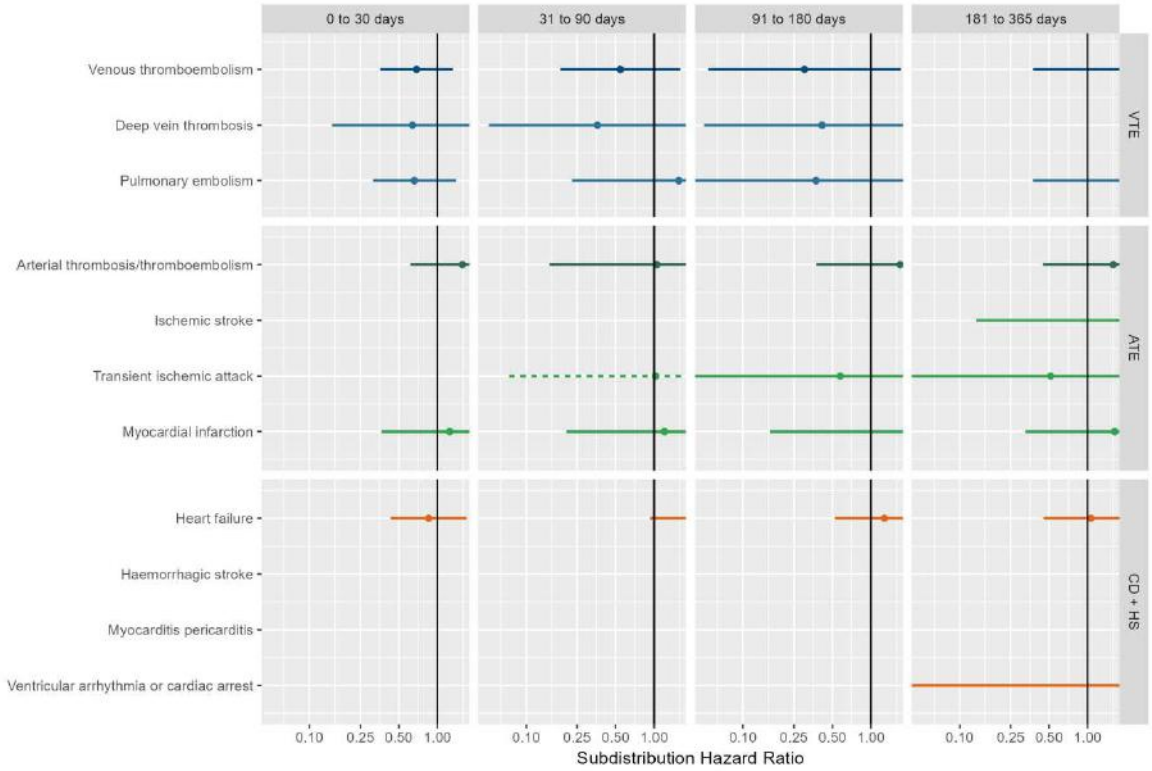
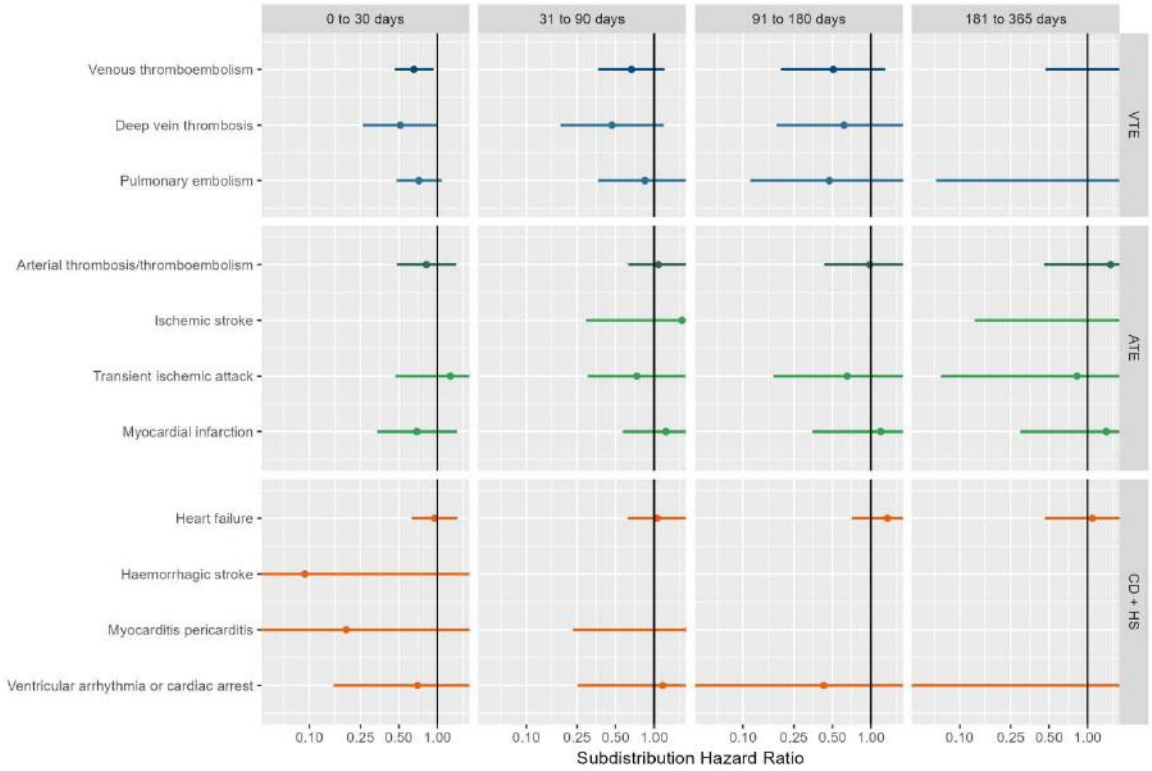


Figure S41: Forest plots for comparative effect, meta-analysis across cohorts and databases. Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.



VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S42: Forest plots for comparative effect, meta-analysis across cohorts and databases. Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.



VTE = Venous thromboembolism, ATE = Arterial thrombosis/thromboembolism, CD + HS = Cardiac diseases and Hemorrhagic stroke

Figure S43: Forest plots for comparative effect on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

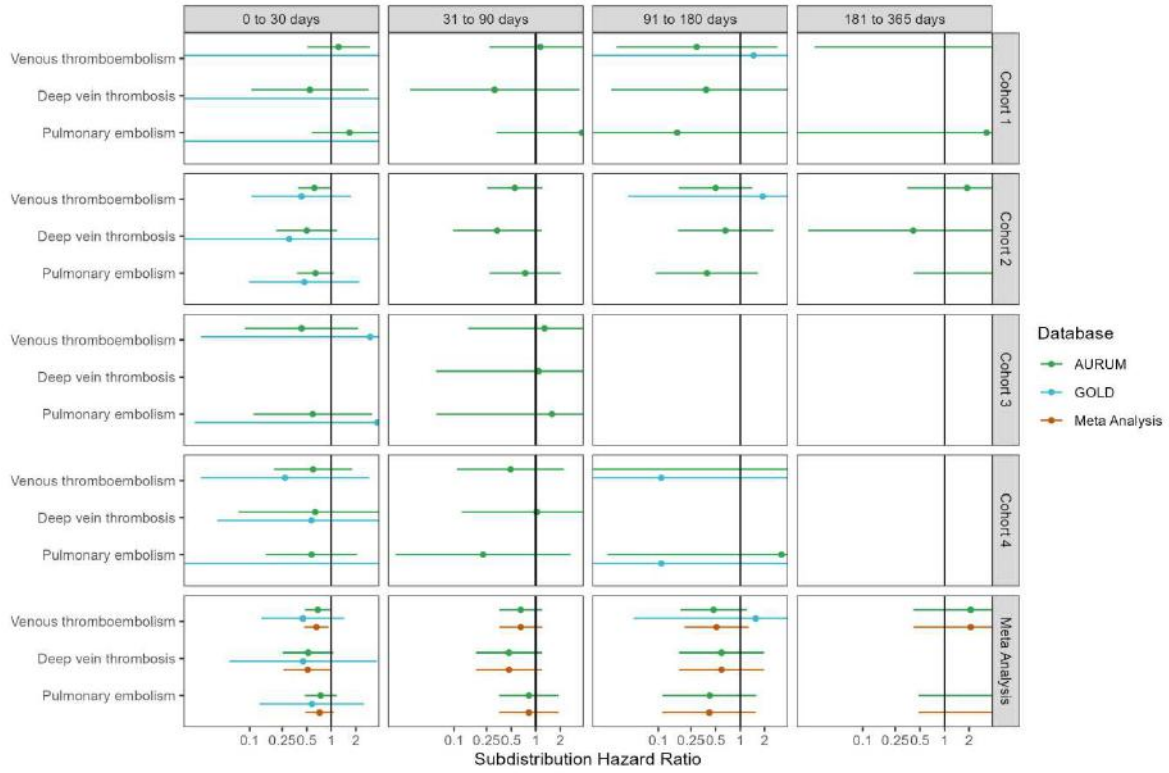


Figure S44: Forest plots for comparative effect on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

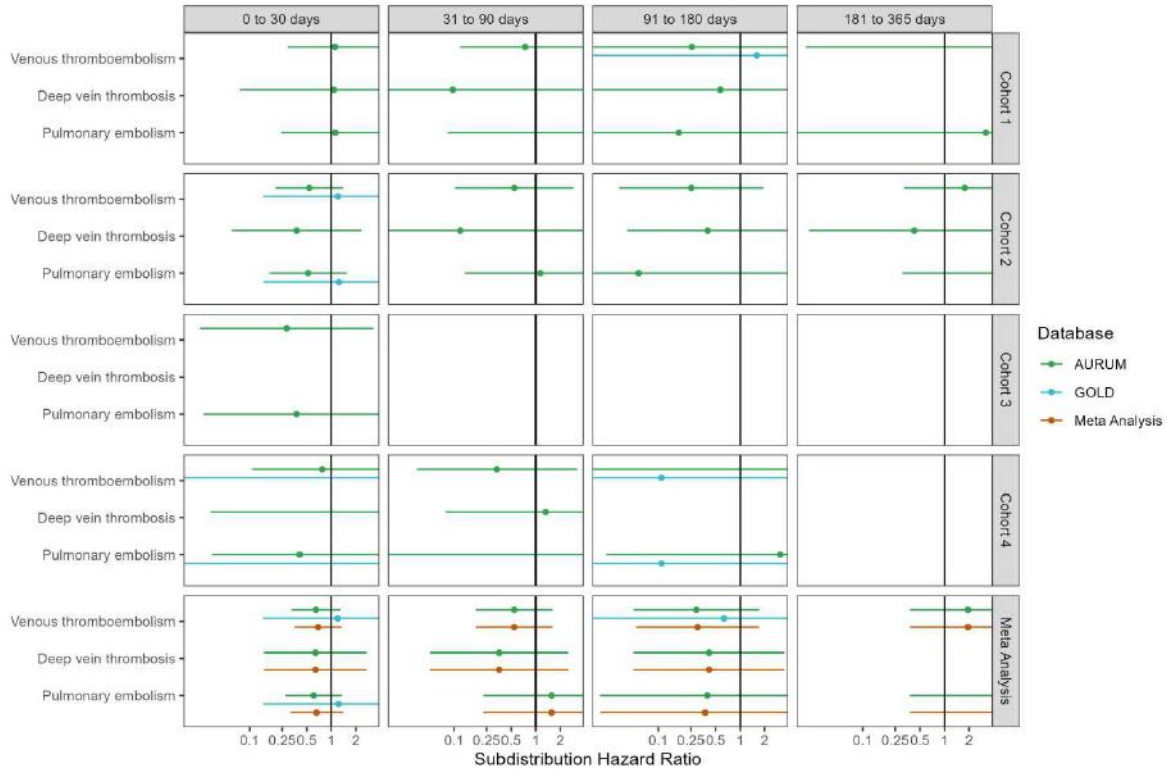


Figure S45: Forest plots for comparative effect on preventing venous thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

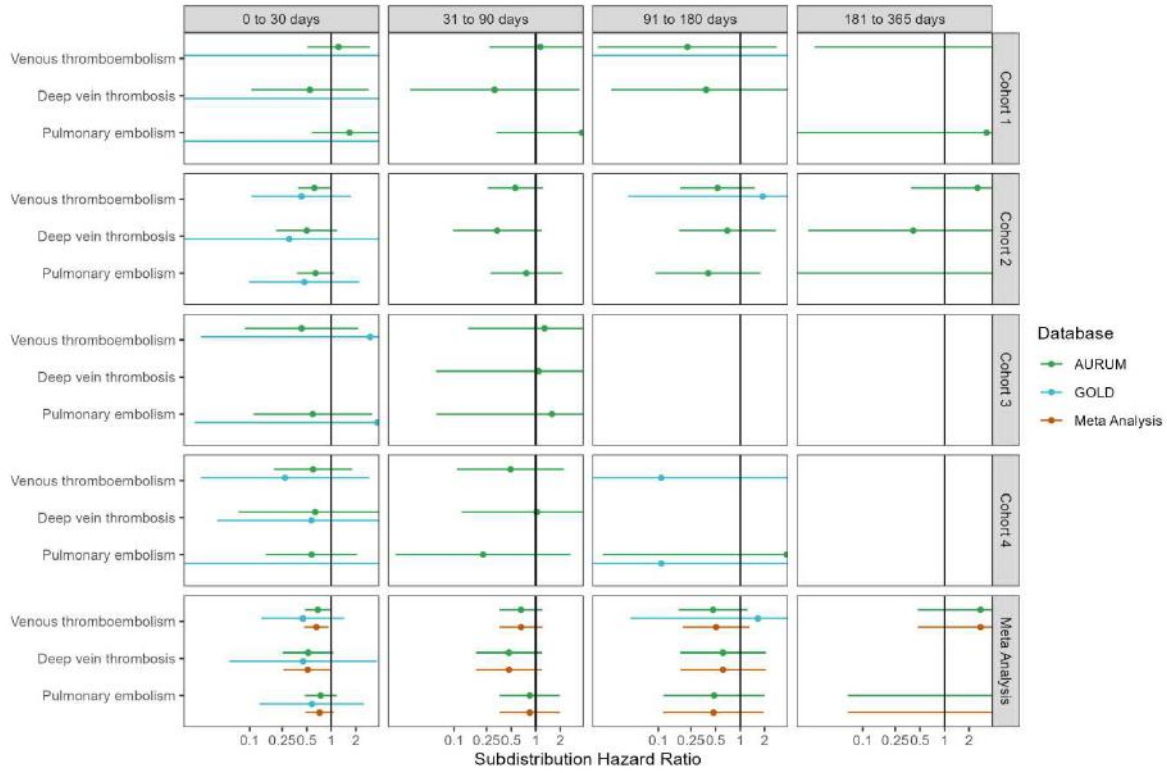


Figure S46: Forest plots for comparative effect on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

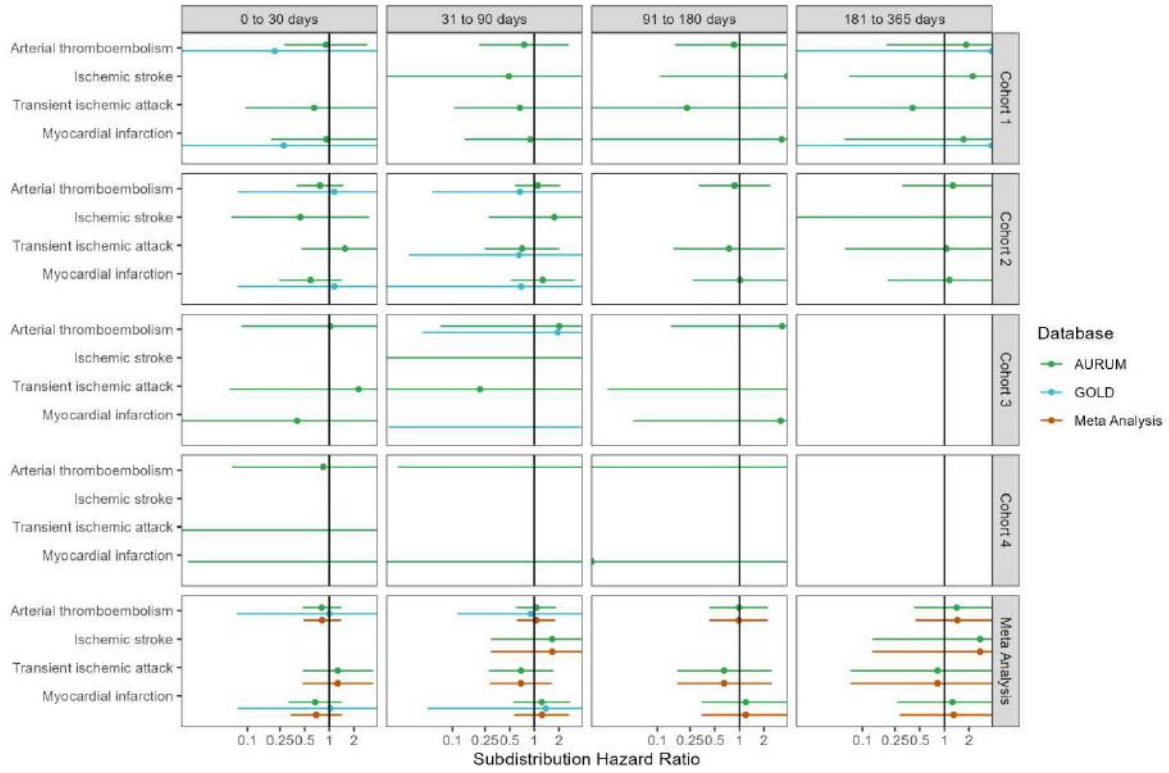


Figure S47: Forest plots for comparative effect on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

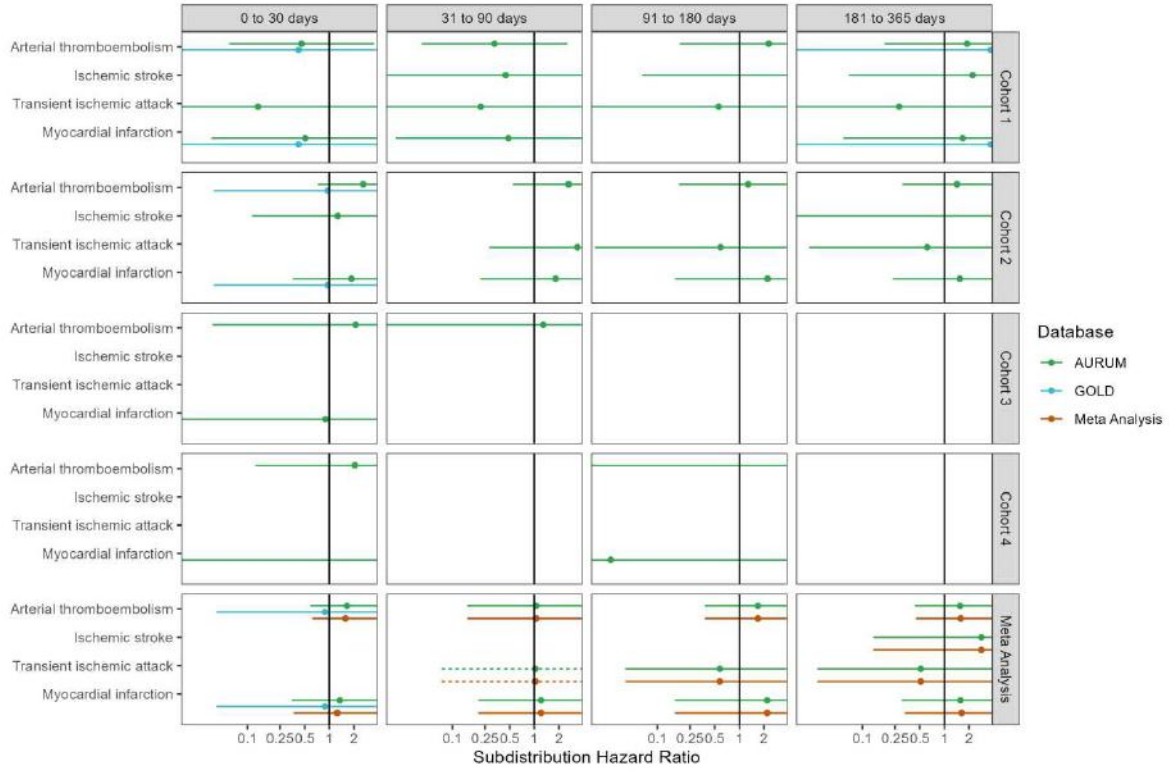


Figure S48: Forest plots for comparative effect on preventing arterial thrombosis/thromboembolism complications, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.

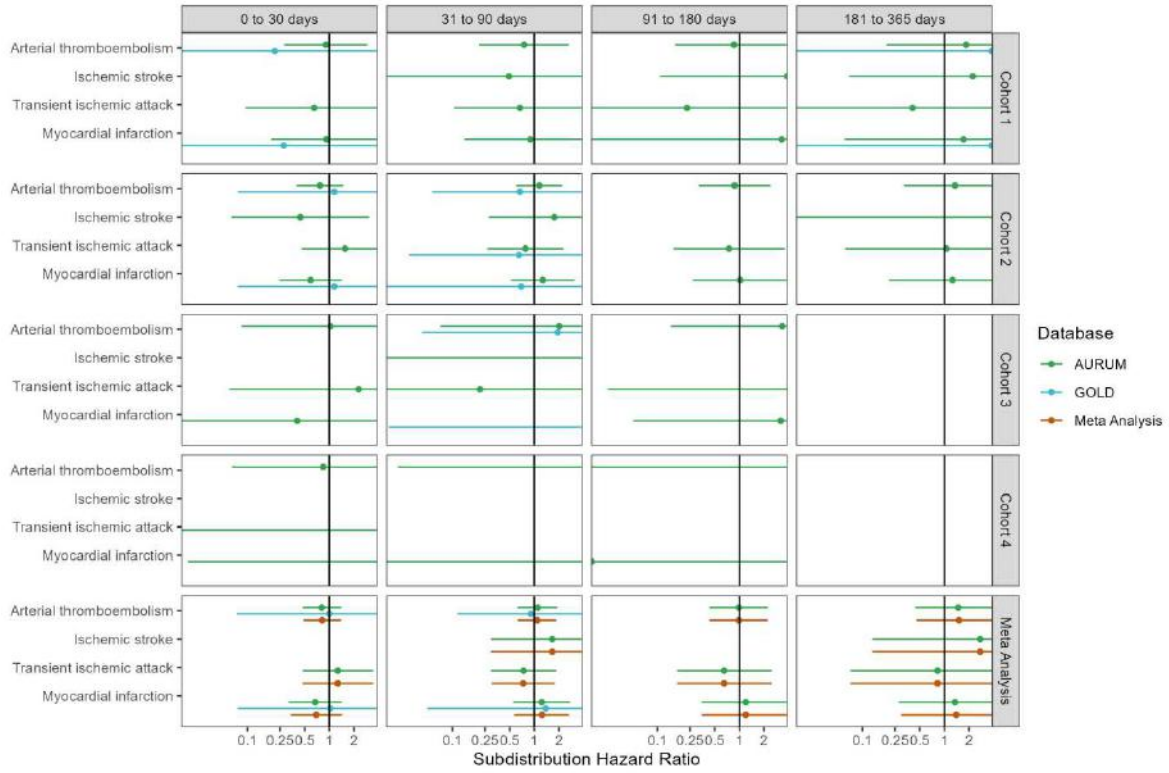


Figure S49: Forest plots for comparative effect on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel). Dashed line represents a level of hetereogeneity I2 > 0.4.

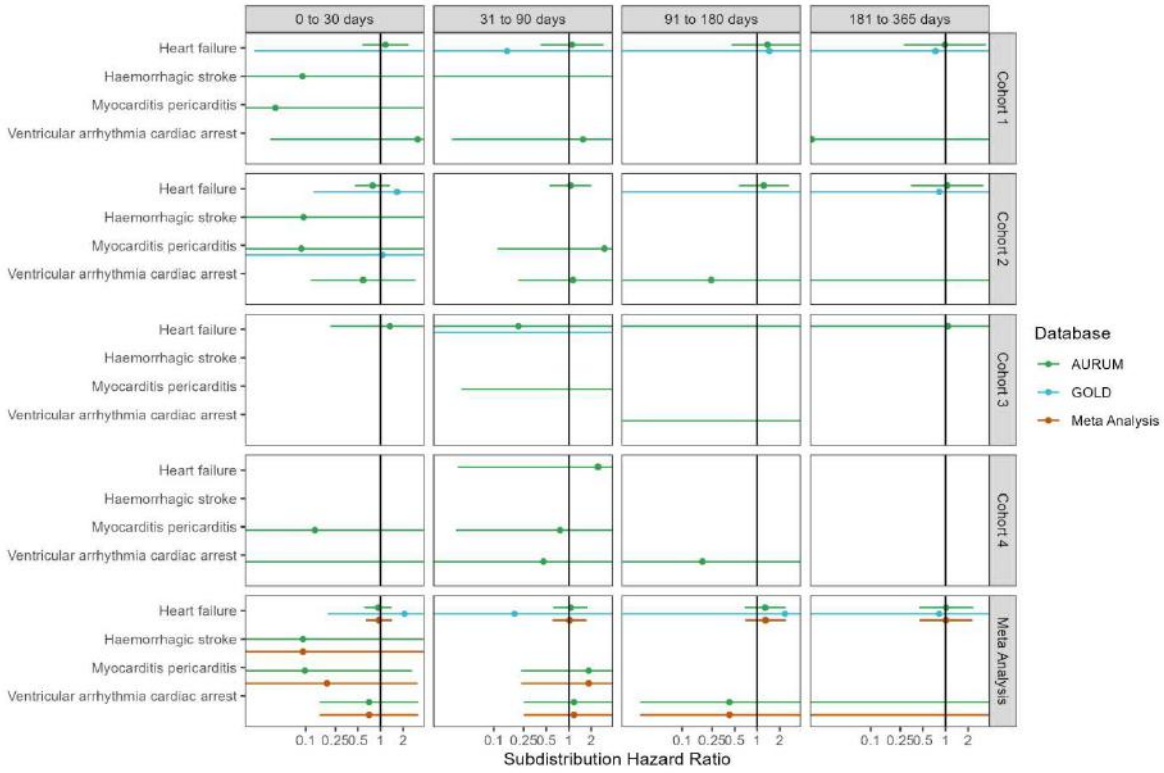


Figure S50: Forest plots for comparative effect on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Follow-up ends at first vaccine dose after index date. Dashed line represents a level of hetereogeneity I2 > 0.4.

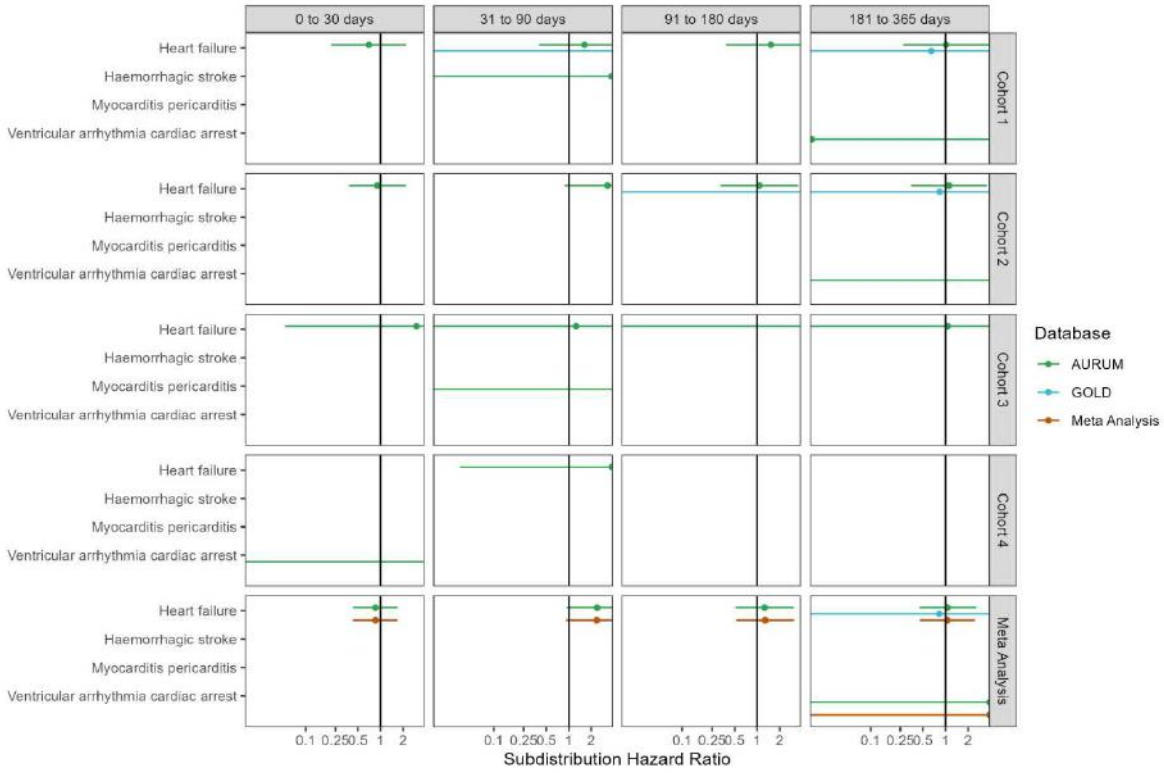
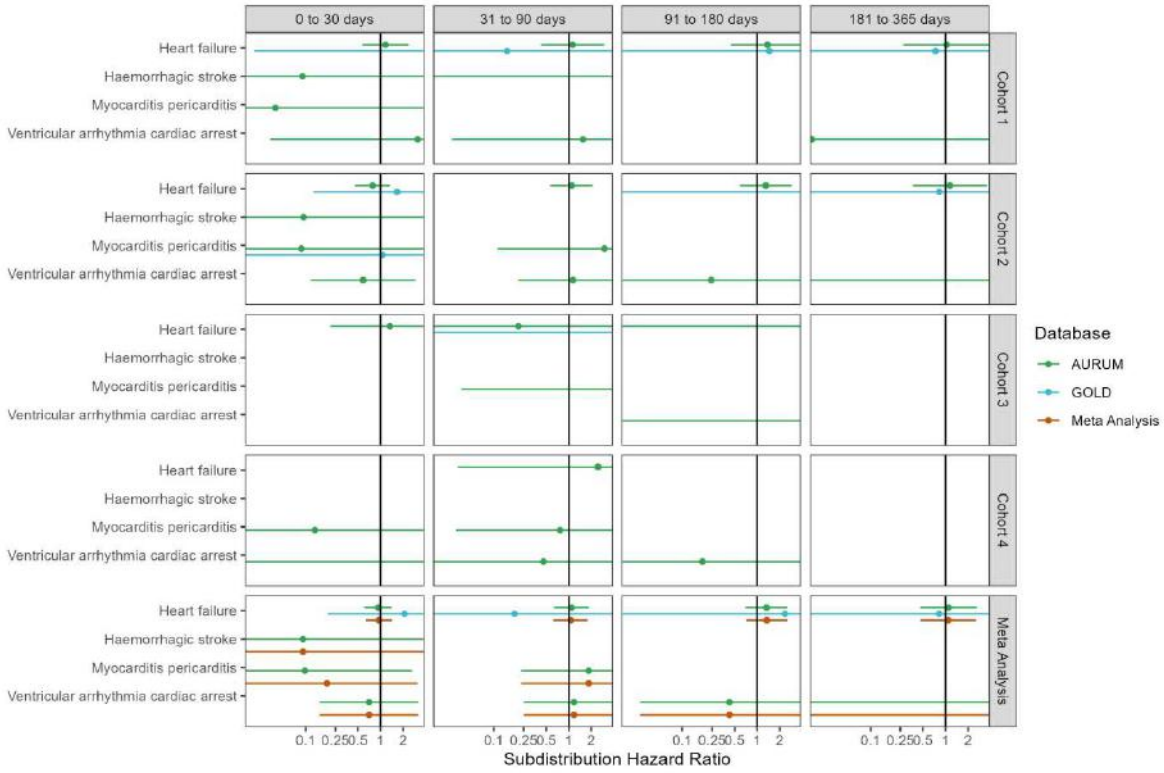


Figure S51: Forest plots for comparative effect on preventing cardiac diseases and hemorrhagic stroke, for each cohort and database (meta-analysis estimates across cohorts in the last panel).Only first outcome after COVID-19 captured. Dashed line represents a level of hetereogeneity I2 > 0.4.



This is Exhibit J referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Medical Complications: a lot of things can be true at the same time

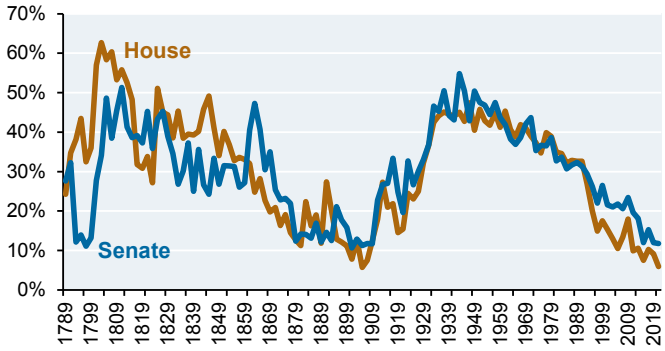
"*The truth is rarely pure and never simple*" – Oscar Wilde

Our 2024 Outlook was released on January 1st and can be accessed at the link above. Markets are pricing in very soft landing outcomes as the Fed pivot was largely reflected in Q4 rallies in stocks and bonds. Meanwhile, the economic spillover in the Middle East has broadened: 12%-15% of world trade passes through the Red Sea, including 30% of global container traffic. More than 100 ships have already been diverted around South Africa, causing shipping rates from Asia to North America to rise by 75% over the last month; Flexport expects them to increase by an additional 50%-100% by the end of January. Most large shipping groups have suspended Red Sea operations, leading to a 1.3% decline in world trade in December. But the overall inflation impact is likely to be modest given very high inventory to sales ratios, deflation in e-commerce, cooling global demand for goods and plenty of capacity in the logistics industry (record containership deliveries expected in 2024).

The rest of this note is about all the things that can be true at the same time. The collapse of the political middle in Congress should not be an excuse for everyone else to abandon the ability to believe things that may appear contradictory, but which are all part of a more complicated reality.

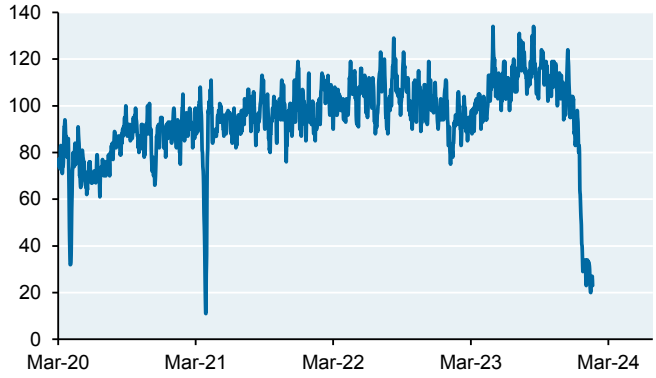
Michael Cembalest, JP Morgan Asset Management

The extinction of political moderates in Congress
% of moderate members in both chambers



Source: JPMAM, Voteview.com, data through 117th congress ending in 2023. Moderates defined as Voteview scores from -0.25 to +0.25

Suez Canal container ship crossings
Number of vessels, 7 day rolling total



Source: Bloomberg, JPMAM, January 22, 2024

Venture capital, office-to-residential conversions and Chick-Fil-A

Last week I presented to venture capital clients in San Francisco and to real estate clients in Utah. Commonly stated themes from VCs: 2020-2021 vintages are still highly challenged and will be subject to further writedowns; the next five years of VC investing will be a golden era for GPs/LPs due to AI/LLM applications; and the decline in Wall Street research coverage of small cap companies is a main reason why small cap IPOs are harder to execute. There are other factors at work in the latter (2003 Wall St research settlement, a move away from soft dollar commissions, Sarbanes Oxley and the SPAC debacle), but I agree in principle.

Real estate developers agreed that office-to-residential conversions are hard to do without massive discounts. But more may be coming: Blackstone defaulted on a building at 55th and Broadway after L Brands vacated 70% of the space, and its appraisal is now down 70% from 2014 levels. In Salt Lake City I also had a Chick-Fil-A chicken sandwich for the first time. It was quite good.

All of these things can be true at the same time.

The costs of Covid lockdowns in the US will linger for years and perhaps decades

In the 1976 movie ***Logan's Run***, the world is turned upside down to preserve the younger generation at the expense of the older one. In 2020-2021, one could argue that the opposite approach was taken in real life. School closures took 50 million children out of class and may prove to be the most damaging disruption in the history of American education, setting student progress in math and reading back by two decades and widening the achievement gap separating the rich and poor[1]. This cohort of children is projected to experience diminished lifetime earnings and be a drag on growth. Lockdowns also resulted in increased domestic violence, loneliness, depression and anxiety[2]. Hazardous alcohol use and dependence increased for those under lockdowns compared to those not under restrictions[3]. Stanford estimates that chronic absenteeism rose from 15% before the pandemic to 25+% at a national level in 2021-2022 and doubled in states like California in 2022-2023.

The FDA has recalled major drugs and has issued black box warnings on others

Substantial risks are sometimes not discovered until well after completion of trials and FDA approval.

RECALL

Drug	Manufacturer	Year	Reason
Bextra	Pfizer	2005	Heart attack and stroke
Vioxx	Merck	2004	Coronary heart disease
Baycol	Bayer	2001	Rhabdomyolysis (muscle disorder)
Phenylpropanolamine	Multiple manuf.	2000	Cardiac events and stroke
Rezulin	Warner-Lambert	2000	Sudden liver failure
Posicor	Roche	1998	Mortality
Fen-Phen	Wyeth-Ayerst	1997	Heart disease
Seldane	Hoescht Marion	1997	Cardiac arrhythmia
Diethylstilbestrol	~300 manuf.	1971	Rare cancers in offspring

BLACK BOX WARNING

Drug	Manufacturer	Year	Reason
Singulair	Merck	2020	Mental health side effects
Tygacil	Pfizer	2013	Increase in all-cause mortality
Lamictal	Glaxo	2010	Skin and organ disorders
Remicade	Janssen Biotech	2009	Infection risk
Actos	Takeda Pharma	2007	Heart failure
Depakote	Abbott Labs	2006	Birth defects
Prozac & Zoloft	Eli Lilly & Pfizer	2004	Mental health side effects

Source: FDA, JPMAM, 2024. The table is not an exhaustive list and shows indicative examples of recalls and black box warnings

Drug company unlawful activity has eroded public perception of the industry

Purdue pled guilty multiple times to criminal charges related to Oxycontin; its $6 billion settlement is now being reviewed by the Supreme Court[4]. Other examples[5]: in 2012, GlaxoSmithKline paid $3 billion to resolve civil and criminal liability related to unlawful promotion of Paxil and Wellbutrin, failure to report safety data on Avandia and civil liability for alleged false price reporting. In 2013, J&J paid $2.2 billion to resolve criminal and civil liability related to Risperdal, Invega and Natrecor. In 2009, Pfizer was fined $2.3 billion for illegal off-label marketing of Bextra and paid to resolve claims related to Geodon, Zyvox and Lyrica. A 2020 JAMA research letter details instances of adulterated drugs, bribery, off-label marketing and kickbacks by drug company[6].

By 2019, the pharmaceutical industry had sunk to the bottom of the Gallup industry poll with a -31 net negative rating, lower than the Federal gov't, oil & gas and law firms. Since then, the industry's net ranking has fallen further to a -42 net negative rating. Its legal issues are affecting these rankings and may explain a decline in medication adherence, difficulty in recruiting clinical trials participants and rejection of effective health interventions, including vaccines[7].

[1] New York Times, November 18, 2023 in its overdue epiphany on the issue of lockdown costs

[2] "*The Causal Role of Lockdowns in COVID-19: Conclusions from Daily Epidemiological, Psychological, and Sociological Data*", Psychiatric Quarterly, Vardi and Lazebnik, June 2023

[3] "*Alcohol dependence during COVID-19 lockdowns*", Killgore et al, Psychiatry Research, 2021

[4] Purdue remains in Chapter 11 bankruptcy. In May 2023, the Court of Appeals for the Second Circuit approved Purdue's bankruptcy plan, overturning the district court's rejection. The plan calls for the Sacklers to contribute $6 bn to satisfy Oxycontin claims **in exchange for a release of third-party claims without having to declare bankruptcy, and after the Sacklers withdrew $11 bn from Purdue**. The Department of Justice appealed the Second Circuit's ruling to the Supreme Court. The Court agreed to hear the case and stayed the Second Circuit's ruling, preventing the bankruptcy plan from being put into effect until the Court rules. Oral arguments occurred a month ago and a ruling is expected this spring

[5] US Department of Justice Press Releases: July 2, 2012 (Glaxo), September 2, 2009 (Pfizer), November 4, 2013 (J&J)

[6] "*Financial Penalties Imposed on Large Pharmaceutical Firms for Illegal Activities*", Arnold et al, JAMA, November 17, 2020

[7] "*Factors associated with public trust in pharmaceutical manufacturers*", Singh et al, JAMA, March 2023

Vaccines have had remarkable success in reducing the frequency of preventable diseases around the world

You wouldn't know it by reading Twitter or listening to sports talk shows, but vaccines are among the greatest achievements in biomedical science and public health. When unchecked, vaccine preventable diseases (VPDs) have enormous social and economic costs in addition to premature deaths they cause. The table shows pre-vaccine incidence of VPDs in the US and 90%-100% post-vaccine declines[8]. In the US, for a single birth cohort, vaccines prevent 20 mm cases of disease and more than 40,000 deaths[9]. Similar results are found in China.

Vaccine preventable diseases in the US

	PRE-VACCINE			POST-VACCINE		
VPD	Annual cases	Annual deaths	Vaccine decade	Cases (2006)	Deaths (2004)	Case decline
Diptheria	21,053	1,822	1940's	0	0	100%
Measles	530,217	440	1960's	55	0	100%
Mumps	162,344	39	1940's	6,584	0	96%
Pertussis	200,752	4,034	1940's	15,632	27	92%
Polio (acute)	19,794	1,393	1950's	0	0	100%
Rubella	47,745	17	1960's	11	0	100%
Smallpox	29,005	337	1790's	0	0	100%
Hepatitis A	117,333	137	1990's	15,298	18	87%
Tetanus	580	472	1940's	41	4	93%

Source: Roush and Murphy, JAMA, 2007

Vaccine preventable diseases: China

VPD	Case decline	by...
Measles	80%	1980's
Pertusis	83%	1980's
Diptheria	81%	1980's
Polio	75%	1980's
Rubella	83%	2010's
Hepatitis A	66%	2010's
Meningitis	78%	2010's
Encephalitis	65%	2010's

Source: Fudan University, 2020

Incidents of vaccine safety problems are extremely rare but have occurred

There have been three safety-related vaccine recalls/withdrawals since the 1950's: polio vaccine safety incident (1955), swine flu vaccine leading to Guillain-Barre syndrome (1976) and the rotavirus vaccine leading to infant bowel obstruction (1998-1999). Other recalls occurred due to testing irregularities in specific vaccine batches.

The CDC has funded research on aluminum content in vaccines and possible connections to childhood asthma[10]. By age 2, the US recommends that children be vaccinated against 15 diseases; aluminum adjuvants appear in seven of them (aluminum is not used in Covid or flu vaccines). While the authors found an empirical association between the degree of vaccine aluminum exposure and persistent asthma, they don't believe their findings constitute strong evidence for questioning safety of aluminum in vaccines. Experts have highlighted other aluminum pathways that were not explored in the study and some data inconsistencies; research is ongoing.

Vaccination trends in the US have been declining, leading to measles outbreaks

Vaccination rates in children fell from 95% before Covid to 93% for the 2021-2022 school year[11]. While a 2% decline doesn't sound like much, the CDC estimates that ~250,000 children are unprotected against measles. In the fall of 2022, Columbus Public Health in Ohio reported 85 cases of measles with 36 children hospitalized; 80 of the 85 infected children had never received a measles-mumps-rubella vaccine[12]. A measles outbreak in Clark County Washington occurred in 2018-2019 affecting 71 people; 86% of the mostly young children infected were unvaccinated[13]. A Kaiser Survey found that 28% of US adults are against mandatory vaccination for children entering kindergarten, up from 16% in 2019[14]. Fifteen states now allow non-medical vaccination exemptions. A 95% vaccination rate is needed to achieve herd immunity against measles; **36 states are below this level**[15].

[8] "*Historical Comparisons of Morbidity and Mortality for Vaccine-Preventable Diseases in the United States*", JAMA American Medical Association, Roush and Murphy, November 14, 2007

[9] "*Simply put: vaccination saves lives*", Proceedings of the National Academy of Science, Orenstein and Ahmed, 2017

[10] "*Association between aluminum exposure from vaccines before age 24 months and persistent asthma at age 24-59 months*", Daley et al, Academic Pediatric Association, August 2022

[11] "*Vaccination Coverage with Selected Vaccines and Exemption Rates Among Children in Kindergarten, 2021–22 School Year*", Seither et al, CDC report, January 13, 2023

[12] Measles Case Summary, Central Ohio Outbreak, February 2023

[13] "*Community Outbreak of Measles - Clark County, Washington, 2018–2019*", CDC, May 2019

[14] Kaiser Family Foundation KFF COVID-19 Vaccine Monitor, Lopes et al, December 2022

[15] "*Coverage with selected vaccines and exemption from school vaccine requirements*", Seither et al, CDC, November 2023

Original Covid vaccines and Omicron boosters were highly effective in preventing serious illness in 2021 and 2022, but protection waned over time as the virus mutated

The original vaccine and the bivalent booster which targeted Omicron subvariants were highly effective in 2021 and 2022 in preventing serious illness[16]. Commonwealth Fund estimates that vaccines prevented more than 18 mm hospitalizations by December 2022[17]; as a reminder, many emergency room and ICU departments were filled to or above capacity with sick Covid patients before vaccination began. By the end of 2022, vaccine and booster efficacy began to decline after several months. The primary reason: the SARS-Cov-2 virus accumulates mutations 2.5x faster than the influenza A flu virus and 7x faster than other coronaviruses[18].

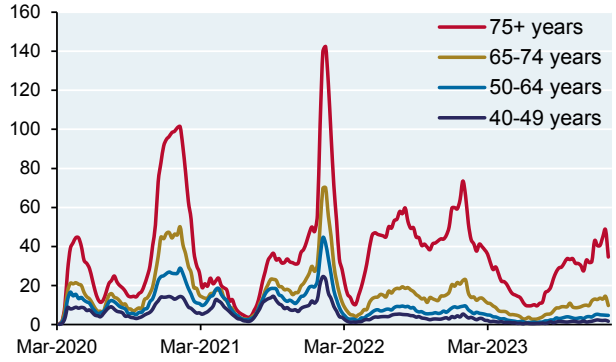
Study timeframe	Efficacy	Doses	Against	Ages	Source	Comments
May 2021 - Jan 2022	79%	1	Hospitalization	Pediatric	Fisman	
May 2021 - Jan 2022	85%	2	Hospitalization	Adolescents	Fisman	
June 2021 - Jan 2022	89%	2	Hospitalization	All	Stoliaroff-Pepin	
June 2021 - Jan 2022	77%	2	Hospitalization	60-75	Stoliaroff-Pepin	
June 2021 - Jan 2022	93%	3	Hospitalization	All	Stoliaroff-Pepin	
Mar 2021 - Mar 2022	78%	3	Hospitalization	All	Bermingham	Declined to 68% after 3 months
Mar 2021 - Mar 2022	88%	3	Hospitalization	65-79	Bermingham	
Mar 2021 - Mar 2022	93%	3	Mortality	All	Bermingham	
Dec 2021 - Aug 2022	68%	3	Hospitalization	All	Link-Gelles (JAMA)	Declined to 36% after 6 months
Jan 2020 - Dec 2022	92%	2	Hospitalization	All	Bacon	Declined to 79% after 7 months
Jan 2020 - Dec 2022	91%	2	Mortality	All	Bacon	Declined to 86% after 5 months
Jan 2020 - Dec 2022	89%	Booster	Hospitalization	All	Bacon	Declined to 71% after 4 months
April 2022 - Mar 2023	76-79%	2nd booster	Hospitalization	All	ECDC	Vs primary vaccination; declined to 43% after 6 months
April 2022 - Mar 2023	76-85%	2nd booster	Mortality	All	ECDC	Vs primary vaccination, declined to 50% after 6 months
Sept 2022 - April 2023	62%	Booster	Hospitalization	18+	Link-Gelles (CDC)	Vs primary vaccination, declined to 24% after 4 months

Sources: see footnotes below

Further mutations have rendered protection from original vaccines and Omicron boosters not that different from being unvaccinated; fortunately, both cohorts benefit from some degree of immunity

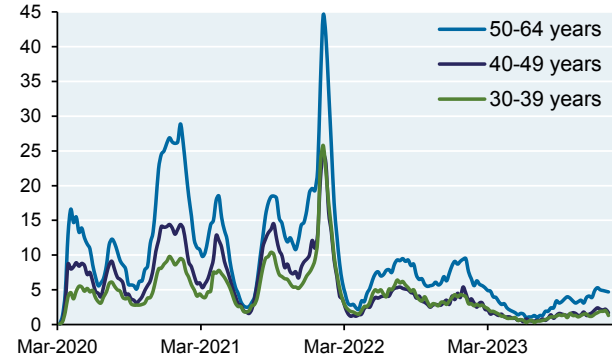
At the end of 2023, the protection against serious illness from the original vaccine and Omicron boosters had declined and was not much different from protection present in unvaccinated, previously infected people[19]. But this framing may overstate the risks to public health. At this point, 99% of people have had at least one vaccine dose, have been infected or have had both an infection and a vaccine[20]; and so far, **both vaccine-induced and acquired immunity from prior infection are holding up well.** As shown below, Covid hospitalizations are much lower than in prior infection waves, particularly for those below the age of 65.

US COVID hospitalizations by age starting at age 40
Weekly rate per 100,000



Source: CDC, JPMAM, January 6, 2024

US COVID hospitalizations by age, 30 to 64
Weekly rate per 100,000



Source: CDC, JPMAM, January 6, 2024

[16] For the table: Fisman, *PLOS One*, March 31, 2023; Stoliaroff-Pepin, *Vaccine*, December 2, 2022; Bermingham, UK Office for National Statistics, June 7, 2023; Link-Gelles, *JAMA*, March 15, 2023; Bacon, *The Lancet*, February 10 2023; European Center for Disease Prevention and Control, November 2023; Link-Gelles, CDC, May 26, 2023

[17] Fitzpatrick (University of Maryland) et al, Commonwealth Fund, December 2022

[18] "*An atlas of continuous adaptive evolution in endemic human viruses*", Kistler and Bedford, November 2023

[19] "*BNT162b2 XBB1.5-adapted Vaccine and COVID-19 Hospital Admissions and Ambulatory Visits in US Adults*", Tartof et al, Kaiser Permanente, December 28, 2023

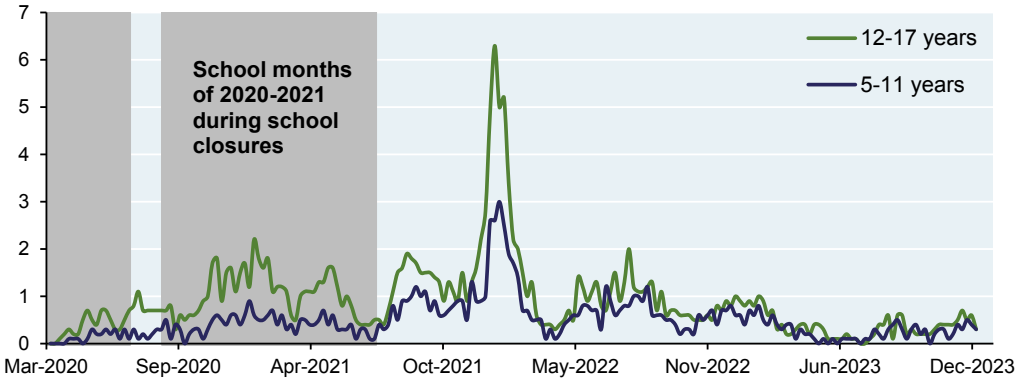
[20] Shane Crotty, Chief Scientific Officer at the La Jolla Institute of Immunology

Hospitalization rates are currently extremely low for school aged children, and were not much higher when schools were closed in 2020 and 2021…

Covid hospitalization rates for school aged children are now less than 1 per 100,000. Note that during the school months from March 2020 to September 2021 when many schools were closed, hospitalization rates were not much higher. When considering lifelong costs that the Covid generation of students incurred (see page 2), I can't imagine how the school closures that took place could be justified.

US COVID hospitalizations by age, school aged children

Weekly rate per 100,000



Source: CDC, JPMAM, January 6, 2024

While the latest XBB booster is not protective against infection…

The XBB booster was released in Sept 2023, targeting XBB 1.5, EG.5 and BA.2.86 strains. The XBB booster doesn't do much to prevent Covid transmission; it might suppress infection risk by 30%-40% in the first month and then its efficacy vs infection declines[21].

…it is still highly effective at preventing serious disease, and more protective than being unboosted…

Individuals who took the XBB booster vaccine in the fall of 2023 had a 68% lower chance of hospitalization than unboosted people[22]. Similarly, the Netherlands reports ~70% XBB booster efficacy rates with respect to risk of hospitalization and ICU admission even for older people[23]. In other words, XBB boosters provide more protection than acquired or induced immunity from prior vaccines/boosters.

…and Covid vaccines/boosters also reduce risks of long Covid, which you do *not* want to get

Long Covid cognitive deficits in patients requiring hospitalization are equivalent to 20 years of aging and are associated with evidence of brain injury and reduced grey matter volume[24]. One vaccine dose reduced long Covid risk by 21%, 2 doses reduced risk by 59% and 3+ doses reduced risk by 73%[25]. Unlike Covid mortality risk, long Covid is not just an issue for older people. In the US and UK, long Covid prevalence is highest among those aged 35-44[26]. Researchers at Cornell believe there may be increased risk of Parkinson's-like symptoms due to Long Covid[27]; after the 1918 influenza pandemic, the frequency of Parkinson's disease increased threefold.

[21] Eric Topol, Ground Truths, December 16, 2023

[22] "*BNT162b2 XBB1.5-adapted Vaccine and COVID-19 Hospital Admissions and Ambulatory Visits in US Adults*", Tartof et al, Kaiser Permanente, December 28, 2023

[23] "*Early COVID-19 vaccine effectiveness of XBB.1.5 vaccine against hospitalization and ICU admission in the Netherlands, October - December 2023*", de Gier (Netherlands Center for Infectious Disease Control), BMJ, December 13, 2023

[24] "*Post-COVID cognitive deficits at one year are global and associated with elevated brain injury markers and grey matter volume reduction*", Michael (University of Liverpool) et al, Nature Portfolio, January 2024

[25] "*COVID-19 vaccine effectiveness against post-covid-19 condition among 589,722 individuals in Sweden: population-based cohort study*", Bygdell (University of Gothenburg) et al, British Medical Association Journal, October 2023

[26] "*Long COVID and Significant Activity Limitation Among Adults*", Ford et al, CDC, August 2023

[27] "*SARS-Cov-2 infection causes dopaminergic neuron senescence*", Yang et al, Cell Stem Cell Journal, January 2024

Covid mRNA vaccines are not riskless…

While instances are rare (2 to 34 cases per million), mRNA vaccinated individuals are twice as likely as uninfected and unvaccinated individuals to experience short-term myocarditis/pericarditis[28]. Prognosis of carditis following mRNA vaccines is generally good with most recovering within a month. Hong Kong implemented a single-dose mRNA policy for adolescents aged 12-17 after which no further carditis cases occurred[29]. Also: myocarditis from getting Covid is more likely than getting myocarditis from the vaccine for all age cohorts except males under 40, and mRNA vaccine-induced myocarditis risk is roughly the same as for people taking flu vaccines[30].

…and Covid "Trojan Horse" vector vaccines (J&J and AstraZeneca) are not riskless either…

In rare instances, the J&J vaccine led to "thrombosis with thrombocytopenia syndrome" (TTS) which involves blood clots and low blood platelets. TTS from the J&J vaccine resulted in 60 cases and 9 deaths out of 60 million vaccines administered, with greatest risks for women aged 30-49[31]. The J&J vaccine is no longer available in the US; blood clot risks also led to restrictions on AstraZeneca's vector vaccine in Europe.

...but blood clot risk from *getting* Covid is greater than blood clot risk from receiving a vaccine

Increased risk of blood clots from receiving a vaccine is ~1.4 per 1,000,000. But blood clot risk from *getting* Covid is much greater: blood clots were a major factor for hospitalized (8%) and ICU (22%) Covid patients, occurring at a rate of over 12,000 per 1,000,000 people who got Covid[32]. In the first week of getting Covid, blood clot risk is 33x normal levels and in some patients was still 2x higher a year later[33].

Most reported instances of cardiac arrest and sudden death are not attributed to Covid vaccines…

An analysis in the American Heart Association Journal *Circulation* did not find increased rates of sudden cardiac death in young people due to Covid vaccines. Causes of sudden cardiac death, including those who experienced it within 30 days of their vaccine, were consistent with prepandemic causes as established by autopsy[34].

…despite all the references to such events on Twitter…

Twitter's decision to no longer analyze Covid tweet content "opened the floodgates for conspiracy theorizing and misinformation" according to Tim Graham at Queensland University, who also believes that "the sudden deaths trope is perhaps the most salient of the false Covid narratives circulating now, and the most dangerous from a public health perspective"[35]. Wired and Fast Company cited a December 2022 CCDH analysis of Twitter Blue posts with the most interactions; of those containing the word "vaccine", 30% featured misinformation[36].

…and in books published by vaccination opponents…

"*Cause Unknown*" was co-published by Children's Health Defense in November 2022 and argues that Covid vaccines caused a spike in sudden deaths. The book's cover shows a 12 year-old boy that collapsed and died at football practice as an example. But: the boy was never vaccinated against Covid and died due to a malformed blood vessel in his brain; no one contacted the family to ask about their son's death or for permission to use the photo; and no one asked to confirm the date of his death, which the book misdated by a year[37]. If there is a connection between vaccines and sudden death or autism, are these the people you would trust to find it?

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[28] "*Risk of myocarditis and pericarditis in mRNA COVID-19-vaccinated and unvaccinated populations: a systematic review and meta-analysis*", Alami et al, British Medical Association Journal, June 2023

[29] "*Benefits v. risks of COVID vaccination: myocarditis and pericarditis*", Carleton et al, Univ. of British Columbia, May 2023

[30] Katelyn Jetelina, University of Texas Health Science Center

[31] "*The Link Between J&J's COVID Vaccine and Blood Clots*", Kathy Katella, Yale Medicine, May 2023

[32] "*COVID vaccination and venous thromboembolism risk in older veterans*", Journal of Clinical Science, Elkin et al, Feb 2023

[33] "*Blood clot risk remains elevated nearly a year after COVID-19*", Merschel, American Heart Association News, Sep 2022

[34] "*Rate and Cause of Sudden Cardiac Death in the Young During the Pandemic/Vaccination*", Basso et al, Circulation, 2023

[35] "*Twitter is a megaphone for Sudden Death vaccine conspiracies*", Lydia Morrish, Wired, January 2023

[36] "*Twitter Blue profiles are already a hotbed of misinformation*", Chris Stokel-Walker, Fast Company, December 2022

[37] AP News, Michelle Smith and Ali Swenson, October 18, 2023 on RFK Jr and Children's Health Defense

…including RFK Jr

When I was 6, I sent a condolence letter to Ethel Kennedy after RFK Sr's assassination. I received a handwritten reply (which I choose to believe came from Ethel) which hung on my wall until I went to college. I like to read RFK Jr's opinions on Ukraine and the military-industrial complex even when I disagree. But as it relates to RFK's statements on vaccines, an October 2023 article in the *Philadelphia Citizen* by Dr. Paul Offit is very revealing. You can decide for yourself: https://thephiladelphiacitizen.org/guest-commentary-a-conversation-with-rfk-jr/

Despite its efficacy vs hospitalization, the XBB booster is not very popular…

As of early January 2024, the CDC reported that only 20% of adults have received the XBB vaccine, and that only 38% of those over 65 received it[38].

…perhaps since politics plays a role in XBB vaccine uptake…

80% of Democrats trust the XBB booster compared to 33% of Republicans; and ~68% of Democrats will either definitely or probably get the XBB booster compared to 25% of Republicans as per a September 2023 survey[39].

…which may explain relative Covid mortality trends as well

Once Covid vaccines became available, mortality trends differed by party affiliation. One example: Yale researchers found that in Florida and Ohio, the all-cause excess death rate among Republican voters (26%) was much higher than among Democratic voters (18%); the two rates were virtually identical before the pandemic began[40]. Another example: as each Congressional District's exposure to conservatism across several political metrics increases (including the VoteView data we've cited elsewhere), that District's Covid mortality rate and its stress on hospital ICU capacity increases as well[41].

And before I forget…

The 2020 *Proximal Origin* paper that dismissed possible Covid lab origins should be officially withdrawn

The infamous March 2020 "*Proximal Origin*" article[42] dismissed the possibility of Covid laboratory origins barely one month into the pandemic. I agree with those who believe it should be officially retracted by its authors due to a highly compromised editorial process[43]. In a 2023 piece in the Intercept, David Relman at Stanford highlights the many problems with *Proximal Origin:* flawed assumptions, unspoken bias, scant data and unjustified reliance on statements by Chinese researchers[44]. *Proximal Origin* became one of the most widely read papers in the history of science; it might also be one of the **worst**.

Michael Cembalest
JP Morgan Asset Management

SARS-Cov-2 research was one of the first real-world applications of Alphafold (biological AI)

When developing a vaccine to neutralize SARS-Cov-2, biologists sought a 3D rendering of the virus proteins. In one of its first real-world applications, Google/Deep Mind's Alphafold program was used for this purpose. Several SARS-Cov-2 proteins were unknown to researchers, so they used AlphaFold to predict their designs. A few months later, scientists used electron microscopes to determine the exact structure of two of the proteins and confirmed that Alphafold predictions were accurate

[38] https://www.cdc.gov/respiratory-viruses/data-research/dashboard/vaccination-trends-adults.html

[39] "*KFF COVID-19 Vaccine Monitor: Partisanship Remains Key Predictor*", Kirzinger et al, September 2023

[40] "*Excess Death Rates for Republican and Democratic Registered Voters in Florida and Ohio During the COVID-19 Pandemic",* Wallace et al (Yale School of Public Health), July 24, 2023

[41] "*Relationship of political ideology of US federal and state elected officials and key COVID pandemic outcomes following vaccine rollout to adults: April 2021-March 2022*", Krieger et al, Harvard School of Public Health, October 2022

[42] "*The proximal origin of SARS-CoV-2*", Kristian Andersen (Scripps), Andrew Rambaut (University of Edinburgh), Ian Lipkin (Columbia), Edward Holmes (University of Sydney) and Robert Garry (Tulane), Nature Medicine, March 17, 2020

[43] "*Why Proximal Origins Must Be Retracted*", Roger Pielke, July 2023

[44] "*Evolution of a theory: Unredacted NIH Emails Show Efforts to Rule Out Lab Origin of COVID*", James Tobias, The Intercept, January 19, 2023

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Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

AN OPEN LETTER TO THE PRESIDENT OF THE UNIVERSITY OF GUELPH

Friday September 17, 2021

University of Guelph
50 Stone Rd. E.
Guelph, ON,
N1E 2G1

Dear Dr. Charlotte A.B. Yates, President and Vice-Chancellor,

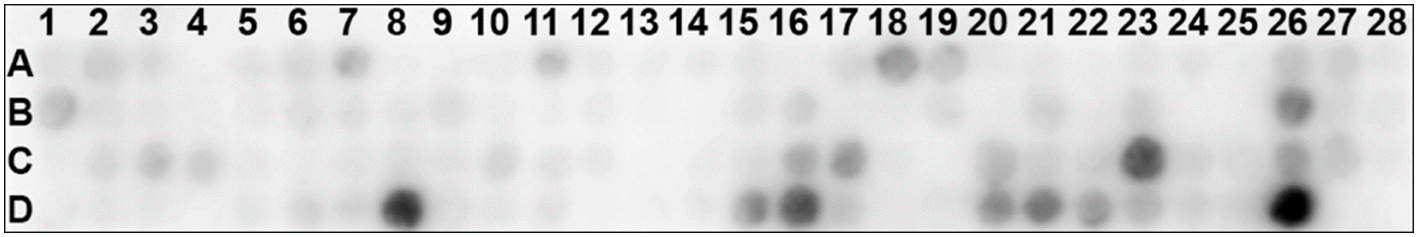
I will forewarn you that this is a lengthy letter. However, it only represents a fraction of the information that I would like to be able to share with you. I have found it necessary to write this so you can fully understand my perspective. With my life and that of my family, many friends and treasured colleagues being destroyed under your watch, I figure the least you can do is read and consider this very carefully. It is incredible to note that many, if not most, of my on-campus detractors have judged me without reading any of my scientific arguments or talking to me about them.

The COVID-19 Vaccine Mandate at the University of Guelph

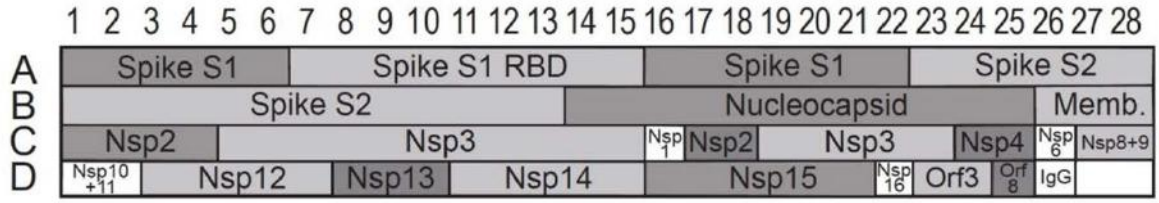
You issued a mandate that everyone within the University of Guelph community must receive a COVID-19 vaccine. I have spent most of my lifetime learning to be a very deep and critical thinker and to follow the weight of scientific evidence. I am a well-recognized expert in vaccinology. As per my extensive funding, research, publication, and teaching records, I am a vaccine lover and an innovator in this field. I promote highly effective vaccines that have undergone extensive, rigorous, and proper safety testing as the most efficient type of medicines that exist. Vaccines that meet these criteria have prevented a vast amount of mortality and morbidities around the world. However, **I could not be in stronger disagreement with you forcing the current COVID-19 vaccines upon everyone** who is part of our campus community. I respect the challenges that a university president faces when trying to manage a large and dynamic academic institution. However, your roots are as a scholar. As a publicly funded institution of advanced learning, it is incumbent on us to demonstrate an ability to view the world around us in a constructively critical fashion such that we can improve the lives of others. We should be able to do this free of political or financial pressures and without bias or prejudice or fear of censorship and harassment. As a viral immunologist that has been working on the front lines of the scientific and medical community throughout the duration of the declared COVID-19 pandemic, I feel compelled to speak on behalf of the many who will not, due to extreme fear of retribution. We now live in a time when it is common practice for people to demand and expect to receive confidential medical information from others. I will not be coerced into disclosing my private medical information. However, for the sake of highlighting some of the absurdities of COVID-19 vaccine mandates I choose, of my own free will, to freely disclose some of my medical information here…

Those with Naturally Acquired Immunity Don't Need to be Vaccinated and are at Greater Risk of Harm if Vaccinated

I participated in a clinical trial that has been running for approximately 1.5 years. The purpose is to develop a very sensitive and comprehensive test of immunity against SARS-CoV-2; in large part to inform the development of better COVID-19 vaccines (https://insight.jci.org/articles/view/146316). My personal results prove that I have naturally acquired immunity against SARS-CoV-2. With this test, spots indicate a positive result for antibodies against a particular part of the virus. Darker spots correlate with more antibodies. Antibody responses correlate with the induction of memory B cells. Antibodies will wane over time, but B cells can survive for many years and rapidly produce massive quantities of antibodies upon re-exposure to a pathogen. On the following page are my results, along with a map of which part of the virus each spot represents…



Peptide Identification on CCJ SARS-CoV-2 SPOT peptide arrays



The dark spot at position D26 is the positive control and indicates that the assay worked. My results demonstrate that I have broad immunity against multiple components of SARS-CoV-2, including the spike protein. Importantly, spot B26 shows that I have antibodies against the membrane protein. This protein is not highly conserved across coronaviruses. As such, it provides evidence that I was infected with SARS-CoV-2. Note that I was sick only once since the pandemic was declared. It was a moderately severe respiratory infection that took ~four weeks to recover from. The SARS-CoV-2 PCR test was negative, despite being run at an unreasonably high number of cycles. This suggests that I was one of the many for whom SARS-CoV-2 has proven to be of low pathogenicity or not even a pathogen (*i.e.* no associated disease). There is a plethora of scientific literature demonstrating that naturally acquired immunity against SARS-CoV-2 is likely superior to that conferred by vaccination only. Indeed, it is much broader, which means that emerging variants of SARS-CoV-2 will have more difficulty evading it as compared to the very narrow immunity conferred by the vaccines. Importantly, the duration of immunity (*i.e.* how long a person is protected) has proven to be far longer than that generated by the current vaccines. The duration of immunity for the mRNA-based COVID-19 vaccines appears to be a horrifically short ~4.5 months. I actually wrote a lay article back in February 2021 to explain why a vaccine of this nature would fail to be able to achieve global herd immunity on its own (https://theconversation.com/5-factors-that-could-dictate-the-success-or-failure-of-the-covid-19-vaccine-rollout-152856). This is why places like Canada, the USA, and Israel have found it necessary to roll out third doses. And now there is talk (and a commitment in Israel) to roll out fourth doses (yes, that's four doses within one year). The World Health Organization recognized the value of natural immunity quite some time ago. Unfortunately, in Canada and at the University of Guelph, we have failed to recognize that the immune system works as it was designed to. Its ability to respond is not limited solely to vaccines. Here are some references to support this: https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-Natural_immunity-2021.1; https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiab295/6293992; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7803150/. As someone who develops vaccines, I can tell you that it is difficult to make a vaccine that will perform as poorly as the current COVID-19 vaccines. Indeed, most vaccines given in childhood never require a booster shot later in life. The take-home message here is that people like me, who have naturally acquired immunity, do not need to be vaccinated. Nor is it needed to protect those around the person who already has immunity. Worse, research from three independent groups has now demonstrated that those with naturally acquired immunity experience more severe side-effects from COVID-19 vaccines than those who were immunologically naïve prior to vaccination (https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(21)00194-2/fulltext; https://www.medrxiv.org/content/10.1101/2021.04.15.21252192v1; https://www.medrxiv.org/content/10.1101/2021.02.26.21252096v1). In other words, **for those with natural immunity, vaccination is not only unnecessary, but it would put them at enhanced risk of harm. Knowing this, nobody should ever mandate COVID-19 vaccination**. Instead, it would be in the best interest of helping everyone make the most informed health decisions for themselves to make voluntary testing for immunity available.

Testing for Naturally Acquired Immunity was a Viable Option but was Ignored

You and the provost met with me and two other colleagues back in March 2021 and we presented the opportunity for the University of Guelph to show leadership and offer testing for immunity to our campus community in support of a safe return to in-person teaching and learning. You embraced this idea with enthusiasm and promised to move forward with it. This did not materialize so one of my colleagues contacted you. Once again, you agreed it was an excellent idea and that you would move forward with it. Nothing happened. So, my two colleagues and I met with one of our vice-presidents in May 2021. They also thought that making an antibody test available was an excellent idea and promised to work on getting it implemented on campus. Nothing materialized. They were contacted again by one of my colleagues. There was no response. There is no excuse for forcing vaccines on people, especially after having been given the opportunity to implement testing for immunity and refusing to do so.

The University of Guelph won't pay for me to receive a booster vaccine against rabies unless I can demonstrate that my antibodies are below what has been deemed to be a protective titer. This is because it would not be appropriate to give me a vaccine that is not without risk if I don't need it. Also, the university does not want to pay the ~$850 cost of the vaccination regimen unless I absolutely need it. In short, you will not allow me to receive that booster vaccine without first evaluating me on an annual basis for evidence of immunity (or lack thereof). So why was this principle rejected for the SARS-CoV-2 vaccines, for which there is vastly less reliable safety data available, and none for the long-term? Canada should have been acquiring data about immunity starting a long time ago. It is a particularly poor precedent for a university to reject the concept of acquiring data that could inform safer and more effective COVID-19 policies. Immunity testing would even benefit vaccinated individuals. It is well known that responses to vaccines in outbred populations follows a normal curve and includes individuals that are non-responders (*i.e.* they are left without immunity and are, therefore, unprotected following vaccination) and low-responders (insufficient protection). In fact, this concept has been the focus of an internationally recognized research program on our campus that has brought many accolades and awards to our institution.

You have banned me from campus for at least the next year. I can show proof of immunity against SARS-CoV-2 but you will not allow me to enter buildings. But someone else can show a receipt saying that someone saw two needles go into their arm and you will allow them to enter. You actually have no idea if that person has immunity. There have even been reported cases of people accidentally or even intentionally (*e.g.* a case in Germany) being administered saline instead of the vaccine. **Does it make sense to ban someone who is immune from campus but allow people who are presumed, but not confirmed, to be immune?** This is a scenario that you have created. As a fellow academic, **I am requesting that you provide me with a strong scientific rationale why you are allowing thousands with an unconfirmed immunity status onto our campus, but you are banning people like me who are known to have immunity**. Further, **please explain how you feel it is ethical to force COVID-19 vaccines on people who are uncomfortable with being coerced when you do not know their immunity status**. Despite attempts to halt the spread of SARS-CoV-2 via masking and physical distancing, the reality is that the virus has not complied with these attempts to barricade it. Indeed, it has infected many people across Canada, many of whom may not have even realized it because it is not a dangerous pathogen for them. From the perspective of a medical risk-benefit analysis, this is a no-brainer. A medical procedure that adds no value but carries known and still-to-be-defined risks should never be mandated!

The University Back-Tracked on Advice from its Own Legal Counsel

I, along with two colleagues, attended a meeting with one of our vice-presidents in May 2021. In that meeting the legal advice that was provided to the University of Guelph was disclosed. We were told this included making COVID-19 vaccines voluntary, that nobody on campus should be made to feel coerced into being vaccinated, and that nobody should feel pressured to disclose their vaccination status. On this basis, I was to serve as one of the on-campus faculty contacts for anyone who experienced any of these issues. **Did Canada's laws change during the summer in a way that rendered this legal advice no longer valid?** Now I am having to spend an inordinate amount of time trying to help the many people whose lives have imploded due to the university's vaccine mandate.

I am a Scientist Who is Knowledgeable and Values Integrity Despite What So-Called 'Fact Checkers' Have Claimed

There are many on our campus who repeatedly put my name out to the public with claims that I disseminate misinformation. Not one of these individuals has ever given me the courtesy of a conversation prior to publicly attacking me. None of them will engage me in public discussions of the science to allow people to judge the legitimacy, or lack thereof, of what I am saying. Censorship on our campus has become as prevalent as it is off-campus. My detractors, rather than showing a deep understanding of the science underlying COVID-19 vaccines, continually refer to the so-called 'fact checks' that have been posted about me. Let me tell you some things about the so-called 'fact checkers'. Firstly, they give scientists and physicians of integrity unreasonably short periods of time to respond to their requests for answers. For example, as I write this letter, I have 13,902 unread messages in my inbox and my voice mail is at maximum capacity. I have yet to see a 'fact check' request prior to its expiry, which remarkably, is often within mere hours of an e-mail being sent. This is an unreasonable expectation from a busy professional. Also, many 'fact checkers' lack sufficient expertise. In some cases, 'fact checker' sites have had to rely on postdoctoral trainees in other countries to write responses.

Most of the harassment against me began after 'fact checkers' cherry-picked one short radio interview that I gave to a lay audience. Some have accused me of only giving half the story in that interview. They were most kind; I was only able to reveal ~0.5% of the story. It is unfair to critique a tiny portion of one's arguments that were presented off-the-cuff to a lay audience with no opportunity for me to respond in real-time. For your information, **I have rebutted every single one of the 'fact checks' that I am aware of** in various public interviews. Let me give you one example that some of our colleagues on our campus have repeatedly misused while harassing me in social media…

One of the many issues that I have raised with the vaccines is that should a reasonable concentration of the free spike protein get into systemic circulation, it could potentially harm the endothelial cells lining our blood vessels. I cited this study: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.121.318902. The authors were contacted, and they claimed I had misinterpreted the study. They said that spike-specific antibodies would mop up any spike proteins in the blood, thereby protecting the blood vessels. They argued that this demonstrated that vaccinating people against the spike protein is a good thing. However, the authors are not immunologists and they failed to recognize the limitations of their own study in drawing these kinds of conclusions. Specifically, they did not recognize that in a naïve individual receiving a mRNA-based COVID-19 vaccine, there are no antibodies; either pre-existing in the host, or in the vaccine formulation. In fact, it will take many days for the antibody response to be induced and for titers to begin reaching substantial concentrations. This leaves a large window of time in which any free spike proteins could exert their biological functions/harm in the body before there are any antibodies to neutralize them. Worse, most of the spike proteins should be expressed by our own cells. In that case, the antibodies will target and kill them in a form of autoimmunity. The authors of the paper forgot that their model was in the context of natural infection, where vaccination would precede exposure to SARS-CoV-2. In that case, I agree that there would be pre-existing antibodies that could neutralize spike proteins of viral origin entering the circulation. This was perceived to be one of the 'strongest' arguments used by others to try to discredit me. The reality is that it is completely incorrect and represents an embarrassing misinterpretation by the authors of the original paper and the many 'fact-checkers' that believed them without question.

Criminal Harassment

You have allowed colleagues to harass me endlessly for many consecutive months. They have lied about me, called me many names, and have even accused me of being responsible for deaths. I submitted a harassment claim and your administrators ruled that it did not meet the bar of civil harassment. In stark contrast, I have been contacted by members of off-campus policing agencies who have told me that it exceeds the minimum bar of criminal harassment. I am sorry, but a faculty member can only take so much bullying and see such a lack of adherence to scientific and bioethical principles before it becomes necessary to speak up. Under your watch, you have allowed my life to be ruined by turning a blind eye to on-campus bullying, ignoring our campus principles of promoting mental

well-being and a workplace in which I can feel safe. In addition to this you have banned me from the campus because I have robust, broadly protective, and long-lasting immunity against SARS-CoV-2 but lack a piece of paper suggesting that it was obtained via two injections. Did you see this front page of one of Canada's major newspapers?...

…remarkably, the on-campus COVID-19 policies you are promoting fuel this kind of pure hatred from people, most of whom have not confirmed their own immunity status, against someone like me who is immune to SARS-CoV-2!!! **Does that make any sense?** My workplace has become a poisoned environment where the bullying, harassment, and hatred against me have been incessant. Are you ever going to put an end to the childish and irrational behaviours being demonstrated by our colleagues? I have received thousands of emails from around the world that indicate the university should be embarrassed and ashamed to allow such childish behaviour from faculty members to go unchecked in front of the public. I have invested a decade of my life into the University of Guelph. I have conducted myself professionally and worked to an exceptionally high standard. I have consistently received excellent ratings for my research, teaching, and service. I have received rave reviews from students for my teaching. I have received prestigious research and teaching awards. I have brought funding to our campus from agencies that had never partnered with the University of Guelph in our institution's history. I have brought in ~$1 million-worth of equipment to improve our infrastructure, etc., etc. I am a man of integrity and a devoted public servant. I want to make Canada a better place for my family and for my fellow Canadians. We are a public institution. My salary is covered by taxpayers. This declared pandemic involves science that is in my 'wheelhouse'. Since the beginning, I have made myself available to answer questions coming from the public in a fashion that is unbiased and based solidly on the ever-exploding scientific literature. My approach has not changed. Has some of it contradicted the very narrow public health narrative carried by mainstream media? Yes. Does that make it wrong? No. I will stand by my track record. When Health Canada authorized the use of AstraZeneca's vaccine I, along with two colleagues, wrote an open letter requesting that this vaccine not be used, in part on the grounds that it was being investigated for a link to potentially fatal blood clots in many European countries. I was accused at that time by so-called 'fact checkers' of providing misinformation. Less than two months later, Canada suspended the AstraZeneca vaccination program because it was deemed to be too unsafe as a result of causing blood clots that cost the unnecessary loss of lives of Canadians. More recently, I was heavily criticized for raising concerns in a short radio interview about a potential link between the Pfizer BioNTech COVID-19 vaccine and heart inflammation in young people, especially males. This is now a well-recognized problem that has been officially listed as a potential side-effect of the mRNA COVID-19 vaccines. It was also the subject of a recent Public Health Ontario Enhanced Epidemiological Summary Report highlighting the increased risk of myocarditis and pericarditis to young males following COVID-19 mRNA vaccination. As such, I have a proven track record of accurately identifying concerns about the COVID-19 vaccines.

A Lack of Safety Data in Pregnant Females as Another Example of Why Vaccines Should Not be Mandated

I would like to give another disconcerting safety-related example of why a COVID-19 vaccine mandate could be dangerous. We have pregnant individuals or those who would like to become pregnant on campus. There was a highly publicized study in the prestigious *New England Journal of Medicine* that formed the foundation of declaring COVID-19 vaccines safe in pregnant females (https://www.nejm.org/doi/full/10.1056/nejmoa2104983). The authors of this study declared that there was no risk of increased miscarriage to vaccinated females. This study resulted in

many policies being instituted to promote vaccination of this demographic, for which the bar for safety should be set extremely high. Did you know that this apparent confirmation of safety had to be rescinded recently because the authors performed an obvious mathematical error? I witnessed several of my colleagues from Canada and other countries bravely push for a review of this paper under withering negative pressures. Once the editor finally agreed to do so, the authors had no choice but to admit that made a mathematical error. Most of the world does not realize this. This admission of using an inappropriate mathematical formula can be found here: https://www.nejm.org/doi/full/10.1056/NEJMx210016. This means that **the major rationale for declaring COVID-19 vaccines safe in pregnant females is gone! How can someone force a COVID-19 vaccine on a pregnant female when there are insufficient safety data available to justify it?**

Advocating for the Vulnerable and Those Fearful of Retribution

My concern is not primarily for myself. I am using my case to highlight how wrong your vaccine mandate is. I am more concerned for the more vulnerable on our campus. I hold tenure, and if ever there was a time when this was important, it is now. However, I have had to bear witness to numerous horrible situations for students and staff members. Students have been physically escorted off our campus, sometimes being removed from their residence, sometimes with their parents also being escorted off. Staff members have been escorted off campus and immediately sent home on indefinite leaves without pay, leaving them unable to adequately care for their families. In many of these situations it seemed like the interactions intentionally occurred in very public settings with it being made clear to all onlookers that the person or people were not vaccinated. Parents have been denied attending meetings with their children who are entering the first year of a program. They recognize that adult learners would normally not have their parents accompany them, but we are living in unusual times with excessive and unfair (arguably illegal?) pressures being applied and these parents are entitled to advocate and defend the best interests of their sons and daughters. Many students have deferred a year in the desperate hope that our campus community will not be so draconian next year. Others fought hard to earn their way into very competitive programs and are not being guaranteed re-entry next year. Many faculty members refused to offer on-line learning options for those who did not wish to be vaccinated. On the flip-side, there are also faculty members, like many students and staff, who are completely demoralized. This includes some who were happily vaccinated but are upset by the draconian measures of your COVID-19 policies and/or will be unwilling to receive future booster shots. I can tell you many stories of students and staff members who couldn't resist the pressure to get vaccinated because they were losing vast amounts of sleep and experiencing incredible anxiety and were on the verge of mental and/or physical breakdowns. In some of these cases, they were crying uncontrollably before, during, and after their vaccination, which they only agreed to under great duress. This does not represent informed consent! I have had several members of our campus community contact me with concerns that they may have suffered vaccine-induced injuries ranging from blood clots to chest pain to vision problems to unexpected and unusual vaginal bleeding. Can I prove these were due to the vaccine? No. But can anyone prove they were not? No. And it is notable that these are common events reported in adverse event reporting systems around the world. In all cases, the attending physicians refused to report these events, even though it is supposed to be a current legal requirement to do so. These people obediently got vaccinated and were then abandoned when they became cases that did not help sell the current public health messaging.

A World Where Everyone is Vaccinated Looks Nothing Like Normal

The two-week lockdown that was supposed to lead into learning to live with SARS-CoV-2 has turned into the most mismanaged crisis in the history of our current generations. I ask you to look around with a very critical eye. You just reported that 99% of the campus community is vaccinated. Congratulations, you have far exceeded the stated standard for what is apparently the new goal of 'herd vaccination'. I cannot use the typical term 'herd immunity' here because immunity is not being recognized as legitimate; only inferred immunity based on receiving two needles counts. We were told that achieving herd immunity by vaccination alone was the solution to this declared pandemic. This has been achieved on our campus in spades. I sat in on our town hall meetings with our local medical officer of health who confidently told us that the risk of breakthrough infections in the vaccinated was almost zero. Why, then are people so petrified of the unvaccinated. Look at vaccines for travellers going to exotic locations.

These are vaccines of some quality. Travellers take these vaccines, and not only do they not avoid the prospective pathogen, but they happily travel to the location where it is endemic (*i.e.* they enthusiastically enter the danger zone because they are protected). So, what does our campus look like with almost every person vaccinated? Everyone must remain masked and physically distanced. There is no gathering or loitering allowed in stairwells or any open spaces in buildings or outside. People are still being told which doors to enter and exit, when they can do so, where to stand in line, when to move. Incredibly, time restrictions are even being implemented in some eating areas because some students were deemed to be "snacking too long" with their masks off and, therefore, putting others at risk of death. In short, the on-campus COVID-19 policies are even more draconian than they were last year, but everyone is vaccinated. It doesn't seem like the vaccines are working very well when a fully vaccinated campus cannot ease up on restrictions. But, of course, we already know how poorly these vaccines are performing. Based on fundamental immunological principles, parenteral administration of these vaccines provides robust enough systemic antibody responses to allow these antibodies to spill over into the lower respiratory tract, which is a common point at which pathogens can enter systemic circulation due to the proximity of blood vessels to facilitate gas exchange. However, they do not provide adequate protection to the upper respiratory tract, like natural infection does, or like an intranasal or aerosolized vaccine likely would. As such, people whose immunity has been conferred by a vaccine only are often protected from the most severe forms of COVID-19 due to protection in the lower lungs, but they are also susceptible to proliferation of the virus in the upper airways, which causes them to shed equivalent quantities of SARS-CoV-2 as those who completely lack immunity. Dampened disease with equal shedding equals a phenotype that approaches that of a classic super-spreader; something that we erroneously labeled healthy children as until the overwhelming scientific evidence, which matches our historical understanding, clarified that this was not the case. I have been in meetings where faculty have demanded to know who the unvaccinated students will be in their classes so they can make them sit at the back of the classroom! I can't believe that some of my colleagues are thinking of resorting to the type of segregation policies that heroes like Viola Desmond, Rosa Parks, Martin Luther King Jr., Carrie M. Best, and Lulu Anderson fought so hard against so many years ago.

The Exemption Fiasco

With respect to exemptions for COVID-19 vaccines, the University of Guelph provided a number based on creed or religion but then, remarkably, rescinded these. These previously exempt individuals were required to resubmit applications using a more onerous form; many that had been honoured previously were rejected upon re-submission. Many have been rejected since. Based on the reports I have received from many people these rejections of exemption requests were typically not accompanied by explanations. Nor have many been told, despite asking, who it is that sits on the committee making decisions about these exemptions. I would never be allowed to assign marks to students anonymously, nor without being able to justify them. Yet there seems to be a lack of transparency with exemptions and many of these decisions are destroying people's lives; the outcomes are not trivial. Could you please disclose the names of the people serving on the University of Guelph's committee that reviews exemptions? Also, could this committee please provide to applicants, retroactively, comments to justify their decisions? I have even heard it said in recent meetings that a lot of people are happy to hear that exemptions, including some medical exemptions are being denied. Why are our faculty celebrating refusals of medical exemptions for students?

A Lack of Consultation with the Experts on Vaccines

You have stated on numerous occasions that your COVID-19 policies have only been implemented after extensive consultation with local and regional experts. Interestingly, however, you have refused, for some unknown reason, to consult with any of the senior non-administrative immunologists on your campus. I would like to remind you that vaccinology is a sub-discipline of immunology. Notably, all three of us have offered repeatedly to serve on COVID-19 advisory committees, both on-campus and for our local public health unit, which also lacks advanced training in immunology and virology. The three of us have stayed on top of the cutting-edge scientific findings relevant to COVID-19 and meeting regularly with many national and international collaborative groups of scientists and physicians to debate and discuss what we are learning. I think it is notable that the senior non-administrative

immunologists unanimously agree that COVID-19 vaccines should not be mandated for our campus based on extensive, legitimate scientific and safety reasons.

Mandating COVID-19 Vaccines is Criminal

I am no legal expert but have consulted with many lawyers who have told me that these vaccine mandates break many existing laws. Here is one example copied from the Criminal Code of Canada:

Extortion

- ***346 (1)*** *Every one commits extortion who, without reasonable justification or excuse and with intent to obtain anything, by* ***threats****, accusations, menaces or violence* ***induces or attempts to induce any person****, whether or not he is the person threatened, accused or menaced or to whom violence is shown,* ***to do anything or cause anything to be done****.*

In your case, you are demanding that members of our academic community submit to receiving a COVID-19 vaccine against their will (a medical procedure that may very well be unnecessary and carry enhanced risk of harm) or face banishment from the campus. Again, I am not an expert in this area, but I am confident there will be lawyers willing to test this in court. Those responsible for issuing vaccine mandates will need to decide how confident they are that they will not lose these legal battles.

Integrity of Teaching

In this new world where followers of scientific data are vilified, I also worry about my ability to teach with integrity. Unbelievably, the Minister of Health of Canada, Patty Hajdu, told Canadians that vitamin D being a critical and necessary component of the immune system in its ability to clear intracellular pathogens like SARS-CoV-2 is fake news! Do you now that I have taught all my students about the importance of vitamin D (often in the historical context of how it was discovered as being critical for positive outcomes in patients with tuberculosis that were quarantined in sanatoriums). I also teach the concept of herd immunity, with vaccination being a valuable tool to achieve this. I do not teach the concept of 'herd vaccination' while promoting ignorance of natural immunity. There are other basic immunological principles that I teach that have either not been recognized during the pandemic as legitimate scientific principles or they have been altogether contradicted by public health and/or government officials. Will I still be allowed to teach immunology according to the decades of scientific information that I have built my course upon? Or will I be disciplined for teaching immunological facts? There are many attempts to regulate what I can and cannot say these days, so these are serious questions.

Instilling Fear of a Minority Group Breeds Hatred

We live in an era where issues of equity, diversity, and inclusion are supposed to be at the forefront of all discussions at academic institutions. However, you are openly discriminating against and excluding a subset of our community that happens to be highly enriched with people engendered with critical thinking; a quality that we are supposed to be nurturing and promoting. With COVID-19 mandates, an environment has been created on our university campus that promotes hatred, bullying, segregation, and fear of a minority group whose only wrongdoing has been to maintain critical thinking and decision-making that is based on facts and common sense. I have yet to meet an anti-vaxxer on our campus. Everyone I know of is simply against the mismanagement of exceptionally poor-quality COVID-19 vaccines. History tells us that instilling fear of a minority group never ends well. This scenario must be rectified immediately if our campus is ever to return to a safe and secure working and learning environment for all.

Committing to Abolishing the COVID-19 Vaccine Mandate

President Yates, **the favour of a reply is requested**. Not the kind that defers to public health officials, or a committee, or anyone else. Instead, a reply with the scientific rigour expected from a scholarly colleague rebutting each of my comments and addressing each question. Surely, you know the science underpinning COVID-19 vaccines inside and out by now. I strongly suspect that nobody would made a decision that disrupts an entire community and destroys the lives of some of its members without a fully developed rationale that can point to the weight of the peer-reviewed scientific literature to back it up. If it would be easier, I would be happy to have an open and respectful, but public and blunt moderated conversation about your vaccine mandate in front of our campus community; much like in the spirit of old-fashioned, healthy scientific debates. You can have your scientific and medical advisors attend and I will invite an equal number. I am not saying this to be challenging. I honestly think it would be a great way to educate our campus community and expose them to the full spectrum of the science. And, if I am as wrong as my 'fact checkers' say, I would love for them to demonstrate this for my own sake as much as anyone else's. So far, despite hundreds of invitations, not one person has done this in a scenario where I can respond in real-time. You need to understand; all I want is my life back and to be able to recognize my country again. I want to see the lives of the students, staff, and other faculty members that I have seen destroyed be restored again. I want to be able to return to my workplace and not be fearful of being hated or exposed to social, mental, and physical bullying. Instead, I want to be able to turn my talents and full attention back to being an academic public servant who can design better ways to treat diseases and help train Canada's next generation of scientific and medical leaders. I simply cannot know all that I have shared in this letter and have suffered as much as I have and be silent about it. My great uncles and family members before them served heroically in the World Wars to ensure Canada would remain a great and free democracy. I think they would be horrified by what they see in Canada today. Indeed, many of my friends who immigrated from Communist countries or countries run by dictatorships are sharing fears about the direction our country is heading; it is reminding them of what they fled from. Further, mandating COVID-19 sets a scary precedent. Did you know that multiplex tests for both SARS-CoV-2 and influenza viruses are on the horizon, along with dual-purpose vaccines that will use the same mRNA-based technology to simultaneously target SARS-CoV-2 and influenza viruses (https://www.ctvnews.ca/health/coronavirus/moderna-developing-single-dose-covid-19-flu-combo-vaccine-1.5578445). Rhetorically, will the University of Guelph consider masking, distancing, and/or mandating vaccines for influenza in the future? **Please rescind your COVID-19 vaccine mandate immediately**. **It is doing more harm than good**. **Unbelievably, among many other problems, it is even discriminating against those who can prove they are immune to SARS-CoV-2!**

Mandating COVID-19 Vaccines Creates Absurd Situations

In closing, and to highlight the absurdity of mandating COVID-19 vaccines…
President Yates, I have proven to you that I am immune to SARS-CoV-2, but you have banned me from the campus and ruined my life because I don't have a piece of paper saying that someone saw two needles go into my shoulder. You have a piece of paper that says that someone saw two needles go into your shoulder, but you have not proven that you are immune to SARS-CoV-2. However, you are allowed on campus and your life can proceed uninterrupted. **How is that fair?**

Respectfully and in the mutual interest of the health and well-being of **all** members of our community,

Dr. Byram W. Bridle, PhD
Associate Professor of Viral Immunology
Department of Pathobiology
University of Guelph

This is Exhibit L referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Pause slideshow

Visit the NEW site to access programs and resources! (https://uoguelphca.sharepoint.com/sites/GREATatUofG)

Slide 1 of 3

713 COVID-19 Vaccination Policy

Effective Date: September 7, 2021

Revised Date: May 25, 2022

Paused Date: May 1, 2022

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A. PURPOSE AND SCOPE

i. Consistent with its commitment to a safe and healthy work and learning environment for its community, in accordance with its legislative obligations, the University has adopted the following policy related to access to University Locations, as defined. Throughout the COVID-19 pandemic, the University has sought to implement health and safety protocols and policies based on the advice and recommendations from the provincial government, Ontario's Chief Medical Officer of Health, and the Wellington Dufferin Guelph Public Health.

ii. COVID-19 vaccines play an important role in helping protect our community and bringing the pandemic to an end. Requiring faculty, staff, students, contractors, volunteers, and visitors to submit proof of vaccination helps the University work towards the safest possible working and learning environment for everyone. Consistent with its obligations pursuant to the *Occupational Health and Safety Act* (the "*OHSA*") and the University's Environmental Health and Safety Policy the University has taken the necessary step of adopting this policy to outline the expectations of those attending University Locations.

iii. The Council of Ontario Medical Officers of Health has indicated that vaccination against COVID-19 is the single most effective public health measure to reduce the spread of COVID-19. The University is committed to providing a safe and healthy environment for its employees, students, and visitors and to the prevention and elimination of workplace injuries and illness.

iv. In accordance with the University's Environmental Health and Safety Policy:

   a. The standards prescribed in the *OHSA* and prescribed regulations may be exceeded by specific University Safety Policies and departmental procedures for risk management and due

diligence.

b. The University requires that all employees shall regard safety as a priority in all employment related activities and they shall not endanger the health and safety of themselves in the workplace

v. This Policy applies to all faculty, staff, students, contractors, volunteers, and visitors who access University Locations.

vi. In addition to its obligations pursuant to the *Occupational Health and Safety Act*, universities are required to comply with government regulations and any advice, recommendations and instructions issued by the relevant public health officer. Given the evolving nature of COVID-19 and its variants, details regarding mandatory vaccination and additional health and safety measures may change from time to time. Details regarding these requirements will be made available at https://news.uoguelph.ca/covid-19/ (https://news.uoguelph.ca/covid-19/)

B. DEFINITIONS

For the purposes of this Policy, the following definitions shall apply:

i. **COVID-19 Vaccine** – a Health Canada or World Health Organization approved vaccine.

ii. **Employees –** faculty and staff employed by the University of Guelph.

iii. **Fully Vaccinated** – means meeting current Public Health requirements for COVID-19 vaccination including any and all available and recommended associated booster doses for those eligible.

iv. **Individual** – faculty, staff, students, volunteers, contractors, and visitors who want to access University Locations.

v. **Individuals with Exemptions** – Individuals who have received an approved exemption in accordance with section C.2.

vi. **Mandatory Vaccination Requirements** – has the meaning found in section C. 1.

vii. **Non-Compliant Individuals** – Employees or students who are not compliant with the current Mandatory Vaccination requirements.

viii. **Proof of Vaccination** – a written or digital vaccination record of an Individual's COVID-19 current vaccination status.

ix. **University Locations** – all University of Guelph buildings and University managed facilities, outside and inside, including but not limited to those on its main campus in Guelph, Ridgetown campus and

research stations. University of Guelph- Humber will be subject to Humber College requirements and procedures.

C. POLICY

1. Mandatory Vaccination Requirements

i. Since September 7, 2021, the University has required that all Individuals who want to access University Locations be required to abide by the University's Mandatory Vaccination requirements.

ii. Individuals will be required to provide current/updated Proof of Vaccination as directed by the University, prior to coming to University Locations.

iii. Individuals with approved Exemptions may access University Locations if they follow all COVID-19 safety protocols as defined by the University.

iv. Individuals who have not otherwise received an approved exemption are required to be Fully Vaccinated to be eligible to access University Locations.

2. Exemptions

Exemptions from the COVID-19 vaccination requirement may be requested on the basis of medical reasons or other grounds in accordance with the *Ontario Human Rights Code*. The University will provide information on the exemption request process, as appropriate.

3. Employees

i. Employees who **are required to perform their job duties on-site at University Locations based on operational plans or the nature of the work** are required to comply with the Mandatory Vaccination Requirements.

ii. Employees who **are performing their work both on-site and remotely based on operational plans** must abide by the Mandatory Vaccination Requirements and may work remotely if their manager or supervisor provides work that can be performed on the dates the Employee is expected to be on-site.

iii. Non-Compliant Individuals who are **continuing to perform their job duties remotely based on operational plans** may continue to do so. However, Employees must abide by the University's Mandatory Vaccination Requirements when they return to work on-site at University Locations.

iv. Non-Compliant Individuals who **are required by the University to perform their job duties on-site at University Locations** will be placed on an unpaid leave until they comply with the Mandatory Vaccination Requirements.

4. Students

i. Students who are registered for in-person courses are required to comply with the Mandatory Vaccination Requirements.

ii. Non-Compliant Individuals will not be eligible for in-person courses nor be able to attend any University Location. Non-complaint Individuals are eligible for courses offered remotely only.

D. Consequences for Contravention of this Policy

i. Information submitted by Individuals regarding Proof of Vaccination or Exemptions may be randomly audited to confirm that appropriate documentation has been provided.

ii. The University reserves the right to take action with respect to any Individual who contravenes this Policy or who submits fraudulent documentation including but not limited to Proof of Vaccination or documentation supporting an exemption, to the University. Consequences may include but are not limited to:

a. For Employees - removal of access to University Locations and/or disciplinary action, up to and including termination of employment.

b. For students – removal of access to University Locations and/or disciplinary action.

c. For volunteers, contractors, visitors – removal of access to University Locations

E. Privacy

Personal information collected in accordance with the University's Mandatory Vaccination requirements and this Policy will be collected by the University pursuant to Section 11 of the *University of Guelph Act 1964* and consistent with Ontario's *Freedom of Information and Protection of Privacy Act*. This Personal information will be used to determine compliance with the University of Guelph's mandatory vaccination requirements. Personal information will be retained for at least one (1) year and otherwise for a reasonable period of time given the purposes for which it was collected. If an outbreak of COVID-19 takes place which may affect an Individual, their personal information may be disclosed to the Wellington-Dufferin-Guelph Public Health or other applicable Public Health Unit to assist with contact tracing efforts.

Personal information may be aggregated on a fully anonymized basis such that it is no longer personal information, and such aggregated anonymized information may be shared by the University with the Ministry of Colleges and Universities, the community, and stakeholders in relation to the University's response to the COVID-19 pandemic including the University's reinforcement of existing public health measures and the University's own efforts to keep our community safe.

F. Duration

This Policy may be reviewed and amended from time to time.

The University has the right to change, modify or revoke this Policy, including by enhancing protections in place and implementing supplementary policies which may be applicable to specific buildings, facilities, or activities, at any time.

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(http://www.linkedin.com/shareArticle?mini=true&url=https://www.uoguelph.ca/hr/staff-faculty-hr-policies-support-staff/713-covid-19-vaccination-policy&title=&summary=)
(/hr/print/8183)

Frequently Used Links

- Benefits (//www.uoguelph.ca/hr/staff-faculty/benefits)
- Employee Groups and Agreements (//www.uoguelph.ca/hr/hr-services-staff-relations/employee-groups-agreements)
- HR Policies (//www.uoguelph.ca/hr/managers-policies-standards-agreements/hr-policies)
- Job Postings (//www.uoguelph.ca/hr/careers-guelph/current-opportunities)
- Employee Self-Service (https://hr.uoguelph.ca/employeeportal/)
- Sun Life eClaims (http://www.sunnet.sunlife.com/signin/mysunlife/home.wca)
- Wellness@Work (https://www.uoguelph.ca/wellnessatwork/)
- Learning & Development Event Registration Calendar (https://www.uoguelph.ca/learningmatters/eventreg/calendar)

- Lynda.com (https://www.lynda.com/)

HR Units

- Environmental Health & Safety (EHS) (//www.uoguelph.ca/hr/hr-services/welcome-environmental-health-safety)
- Learning & Development (//www.uoguelph.ca/hr/hr-services/learning-development)
- Occupational Health & Wellness (//www.uoguelph.ca/hr/hr-services/occupational-health-wellness)
- Staff Relations (//www.uoguelph.ca/hr/hr-services/staff-relations)
- Total Compensation (//www.uoguelph.ca/hr/hr-services/total-compensation)

HR Contacts

To access the Human Resources Directory please visit our HR Contacts page (//www.uoguelph.ca/hr/hr-services-about-human-resources/human-resources-contacts)

Find us on Social Media

(https://www.linkedin.com/company/university-of-guelph?trk=top_nav_home)

(https://twitter.com/UofGHR)

This is Exhibit M referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Science-Based Medicine
Exploring issues & controversies in science & medicine

Internet & social media Public Health Vaccines

"COVID-19 vaccines are going to sterilize our womenfolk," Take 2

Antivaxxers have long claimed that vaccines, particularly HPV vaccines, can damage the ovaries and cause female infertility. That claim has been resurrected for COVID-19 vaccines. The first example relied on a dubious "similarity" between the SARS-CoV-2 spike protein and a placental protein. This time, it's the lipid nanoparticles attacking the ovaries, echoing very old claims about polysorbate-80. Truly, everything old is new again.

David Gorski on June 7, 2021

Post

Before there were safe and effective COVID-19 vaccines authorized for use, such as the vaccines by Pfizer/BioNTech, Moderna, and Johnson & Johnson here in the US, as well as AstraZeneca in Europe and elsewhere, those of us who have been countering the antivaccine movement for many years now were warning about the sorts of disinformation that antivaxxers would spread about them. We were largely correct, too, but I can't really say that it took any particular brilliance or foresight to have been so correct. We simply knew that there is no truly new trope, pseudoscience, or disinformation in the antivaccine narratives and conspiracy theories; so all we did was to predict the repurposing of tried-and-not-true antivax lies.

And so it came to pass beginning as soon as the vaccines neared approval under an emergency use authorization (EUA) by the FDA that antivaxxers repurposed all their old tropes for COVID-19 vaccines, claiming that they were loaded with "toxins" (the lipid nanoparticles in the mRNA-based vaccines, given that they can't contain aluminum, don't you know?); blaming every death reported to the Vaccine Adverse Event Reporting System (VAERS) database on vaccines, when VAERS is not designed to determine causation and we would expect a large baseline number of deaths in the time periods covered by random chance alone; claiming that vaccines cause Alzheimer's and prion disease; blaming the vaccines for cancer; resurrecting the favorite old trope of "shedding" from the vaccinated in the most risible manner possible; invoking evolution to predict the selection of more deadly coronavirus variants that could wipe out humanity; warning that the vaccines can "permanently alter your DNA"; and that they make females infertile. I will admit that there were a couple of new ones, albeit variations on a theme. For instance, because of the new mRNA- and adenovirus-based technologies used to develop the current crop of vaccines, antivaxxers have falsely referred to them as "experimental gene therapy" rather than vaccines, and, because vaccination in the shoulder can lead to transient inflammation of the lymph nodes under the arm, which has led to some unnecessary biopsies after mammography for breast cancer screening, antivaxxers have tried to claim that the vaccines cause breast cancer. So I guess I should say that there's almost nothing new under the sun.

This is why I was not particularly surprised to see the "toxins" gambit with respect to COVID-19 vaccines rear its ugly head again, in particular with respect to the lipid nanoparticles in the vaccines. I was, however, slightly impressed with how antivaxxers had combined it with the "vaccines cause sterilization" trope again. (Or maybe they were combining the "vaccines are sterilizing our women" trope with the toxins gambit. I guess it doesn't matter that much which is the case.) Since I keep seeing the study that antivaxxers mangle coming up again and again and again on antivaccine social media, I decided that I had to address this new marriage of two antivaccine tropes. Let's just say that they're two crappy tastes that taste crappy together.

It begins, as is often the case, on Twitter

I first recall seeing the antivaccine narrative, claiming that lipid nanoparticles from COVID-19 vaccines accumulate in the ovaries and other tissues, showing up on Twitter from “Nurse Erin”:

NurseErin
@erin_bsn · **Follow**

There is evidence to suggest that the Covid mRNA-LNP (lipid nanoparticles) are adhering themselves to human organs (i.e. female ovaries). No long-term studies. You can never get unvaccinated. Leaked confidential study from Pfizer. files.catbox.moe/0vwcmj.pdf #knowledgeispower

12:12 PM · May 30, 2021

824 **See the latest COVID-19 information on X**

Read 48 replies

Conspiracy theorists being conspiracy theorists, “Nurse Erin” says that the claim that lipid nanoparticles from the Pfizer vaccine “adhere” to the ovaries is based on a “leaked confidential” study from Pfizer (of course). It turns out that “Nurse Erin” is an antivaccine nurse named Erin Marie Olszewski, who caused a minor ruckus last summer after having worked as a traveling nurse during the first surge of the pandemic last spring. She wrote a book about it, *Undercover Epicenter Nurse: How Fraud, Negligence, and Greed Led to Unnecessary Deaths at Elmhurst Hospital*. (That longtime antivaccine activist J.B. Handley, who has more recently—and not unexpectedly—joined the COVID-19 conspiracy theory antimask grift train, wrote

the foreword should tell you all you need to know about this book, as should the endorsement by Joe Mercola.) In it, Ms. Olszewski claimed that people who had tested negative for COVID-19 were being diagnosed as having COVID-19 anyway, put on ventilators, and "drugged up with sedatives". In the process, besides spinning conspiracy theories, she also appears to have engaged in what sounds like a massive violation of HIPAA by videotaping medical records, and including them in a conspiracy video with minimal redaction. Unsurprisingly, doctors and nurses at Elmhurst understandably felt betrayed and took pains to debunk "Nurse Erin's" disinformation.

In any event "Nurse Erin" appears to have gotten her "information" from here:

fly
@dankdly111 · **Follow**

To be clear this is a Japanese study that was acquired from a group of doctors including Dr. Byram Bridle from the Japanese government.

The EMA report is public knowledge that was released in February of 2021 confirming accumulation in organs.

ema.europa.eu/en/documents/a...

9:12 PM · May 29, 2021

160 Reply Share

Read 4 replies

fly · May 30, 2021
@dankdly111 · **Follow**
Replying to @dankdly111

But all the vaccine proponents told me these reactions are simply 'allergic reactions'

"we hypothesized that this mRNA-LNP platform's adjuvant activity could stem from the LNPs' inflammatory properties...is highly inflammatory [14]. "

fly
@dankdly111 · **Follow**

So as a summary, my research has now revealed to me:
1) They absolutely accumulate in organs
2) They are highly inflammatory
3) Safety studies are suggesting the cause of side effects are primarily from the lipid nanoparticles
4) There is no long term analysis on what happens

9:03 AM · May 30, 2021

88 Reply Share

Read 6 replies

Amusingly, fly tried to appear “reasonable”:

fly
@dankdly111 · **Follow**

Please chill out with the sterilization claims. We don't know for sure. The study points to no known effect of toxicity on ovaries.

inflammatory. So are we sure? How do we know for certain? I'm scared of the answer though.

10:37 PM · May 29, 2021

14 Reply Share

Read 4 replies

But antivaxxers were having none of it:

■ **Doorn** ■ · Jun 2, 2021
@top_grafisch · **Follow**
Replying to @top_grafisch

De gevaccineerde mensen dragen iets over aan de niet-gevaccineerden.
Blijf weg van gevaccineerde!

In geen enkele #COVIDvaccinatie zit een stof die ons beschermt tegen een zogenaamd virus.

gezondwereldnieuws.com/de-gevaccineer…

#coronavaccins
#covidvaccins

gezondwereldnieuws.com
De gevaccineerde mensen dragen iets over aan de niet-gevaccineerden. Blijf weg van gevaccineerde!

■ **Doorn** ■
@top_grafisch · **Follow**

Huiveringwekkende studie #mRNAvaccin nanodeeltjes

Japans onderzoek over #LipidNanoParticles (#LNPs) die de #mRNAcode bevatten, en na #vaccinatie op grote schaal door 't lichaam circuleren en hersenen, milt, dikke darm, hart, lever, longen etc. bereiken

frontnieuws.com
Huiveringwekkende studie toont aan dat mRNA-vaccin nanode...
Nieuw onderzoek, uitgevoerd in Japan, toont aan dat Lipid NanoParticles (LNPs) die de mRNA-code bevatten, na vaccinati...

1:03 PM · Jun 3, 2021

5 Reply Share

Read 2 replies

Which brings me to Byram Bridle, who, if the above Tweets are to be believed, is the person who “discovered” this “confidential” Japanese study. Before that, he had been spreading misinformation about the “deadly spike protein” produced by COVID-19 vaccines:

> **Ossi Tiihonen**
> @OssiTiihonen · **Follow**
>
> Dr Byram Bridle, Professor of Viral Immunolog: The spike protein in the covid vaccines is a very dangerous toxin. This 7 minute video can save your life, your childrens' lives and your grandchildren's lives.

I'll just refer you to my extensive discussion from two weeks ago of the studies being misrepresented by antivaxxers.

Enter Byram Bridle

While I saw narratives based on this same study showing up on Twitter a lot, leave it to Mike Adams to turn up the conspiracies to 11, with an article titled "Horrifying study reveals mRNA vaccine nanoparticles are circulated throughout the entire body: Brain, heart, liver, ovaries, testes and more":

> "Not surprisingly, everything the establishment tells us about covid vaccines has been a calculated lie. One of the biggest and most treacherous lies is that "mRNA vaccine shots stay in the arm and don't circulate nanoparticles around the body." Now we know that is a complete lie, as new research conducted in Japan shows that Lipid NanoParticles (LNPs) containing the mRNA code are widely circulated around the body after vaccination, reaching the brain, spleen, large intestine, heart, liver, lungs and other organs.
>
> The study paper, originally written in Japanese and auto-translated into English, can be found at this link on Natural News servers (PDF).

> study that uses luciferase enzymes and radioisotope markers to accurately track the distribution of Pfizer's mRNA LNPs across the body.

Of course, Adams also cites Dr. Bridle, who, as it turns out, is an associate professor and viral immunologist in the Department of Pathobiology in the Ontario Veterinary College at the University of Guelph, and apparently the main source of this new "variant" (sorry, couldn't resist using the word) of the "toxins gambit." (Dr. Bridle is a rather…appropriate…name for a faculty member at a veterinary college.) As you might guess, he is an antivaxxer, antimasker, and COVID-19 conspiracy theorists and has made a number of false claims about vaccines dating back to the early days of the pandemic. He is also engaged by Elders Without Borders as an expert witness on behalf of Adam Skelly (owner of Adamson BBQ), and has testified against the effectiveness of masks, against lockdowns, and against the "experimental" vaccines (which he reasons are unnecessary with his proposed treatment – ivermectin).

Perhaps Bridle's most famous quote was cited by Adams:

> "In short, the conclusion is ***we made a big mistake***. We didn't realize it until now. We saw the spike protein was a great target antigen. We never knew the spike protein, itself, was a toxin and was a pathogenic protein. So, by vaccinating people, we are inadvertently inoculating them with a toxin, and in some people this gets into circulation. And when that happens in some people, it can cause damage, especially with the cardiovascular system. I don't have time, but many other legitimate questions about the long-term safety there for this vaccine. For example, with accumulating in the ovaries, one of my questions is, "will we be rendering young people infertile, some of them infertile?" So, I'll stop there.

You'll see the first part of that paragraph everywhere, usually represented as a "provaccine" scientist saying, "We made a big mistake." Of course, the hilarious part is the huge mistake Bridle makes in the paragraph above, conflating lipid nanoparticles with the spike protein. (More on that later.)

Most relevant to this post, he has made claims that the spike protein made by vaccines:

- Is horribly toxic. (Wrong.)
- "Has implications for blood donation." (Given the transient, infinitesimal amount of spike protein from

- Has "implications for infants that are suckling." (No, spike protein made by vaccines is detectable transiently in the blood at levels that are at the lower limits of a very sensitive assay, and a number of studies show that the vaccine itself is not passed through breast milk.)
- Causes gastrointestinal bleeding in infants consuming the breast milk of their vaccinated mothers, based on VAERS reports. (Again, anyone can submit a report of any adverse reaction to VAERS, and antivaxxers have been weaponizing those reports, implying causation where there is none—or even a real correlation.)
- Endangers fertility. (Not true, as I discussed before. Spike does not resemble the placental protein syncytin, the previous "mechanism" invoked by antivaxxers when fear mongering about COVID-19 vaccines and fertility. The new "mechanism" of Dr. Bridle's promoted by Ms. Olszewski will be addressed in the next section.)

One also notes, as is often the case for scientists who spread misinformation about COVID-19 vaccines, an undisclosed conflict of interest:

> A second emphasis of the lab is the study of host responses to viruses. An area of focus is developing a better understanding of the mechanisms underlying virus-induced cytokine storms. Dr. Bridle's research team has identified a critical role of signaling through the type I interferon receptor in the negative regulation of an extensive network of cytokines. Cytokine responses to viruses are often very different between females and males and the Bridle lab group is seeking to understand why. At the intersection of these two programs, is a research initiative aimed at modifying the research team's optimized cancer vaccine platforms to target severe acute respiratory syndrome coronavirus (SARS-CoV)-2, which is the causative agent of the coronavirus disease identified at the end of 2019 (COVID-19). The long-term goal is to have a flexible technological platform to rapidly develop vaccines against highly pathogenic coronaviruses that may emerge in the future.

Yes, Dr. Bridle is trying to develop his own vaccine and treatments for COVID-19. Sound familiar? It should. For example, Geert Vanden Bossche, who is also spreading misinformation about COVID-19 vaccines, owns a company that is trying to develop a vaccine based on a technology to activate natural killer cells.

had his own measles vaccine under development at the time he published his case series linking the MMR vaccine to autism in 1998. The idea seems to be to attack current vaccines as dangerous and ineffective, feeding the antivaccine movement, to pave the way for your own vaccine. Indeed, a year ago Dr. Bridle received a $230,000 grant from the provincial government to develop a vaccine using the very same spike protein that he's been demonizing, although that might be the "big mistake" he has been confessing to.

About that biodistribution study

Now that I've established the origin of the antivaccine misuse of the biodistribution study, at least as closely as I can, let's take a look at the claims of Ms. Olszewski, Mr. Adams, and Dr. Bridle themselves and compare them to the actual study, so helpfully stored at so many antivaccine websites. The original report is in Japanese, but there is what appears to be a machine-translated version available.

The first thing to note is that this biodistribution study is in rats, not humans. Wistar Han rats received a 50 µg dose of lipid nanoparticles labeled with ^{3}H, which is radioactive. Radioactivity was measured in various organs at 0.25, 1, 2, 4, 8, 24, and 48 hours post-injection.

The second thing to note is that this study was only of the lipid nanoparticles, not the full vaccine containing the mRNA for the SARS-CoV-2 spike protein. The dose used was 50 µg. The human vaccine contains 0.43 mg ALC-0315=(4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), ALC-0159=0.05 mg 2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.2 mg cholesterol. That is basically ~0.46 mg lipids or 460 µg. Let's just round it up to 500 µg (0.5 mg). That's approximately 10x the dose given to the rats. However, for the typical "70 kg" male, 0.5 mg represents a per-weight dose of 0.0071 mg/kg, or 7.1 µg/kg. Let's compare to the rats, which generally weigh around 200 g (0.2 kg), give or take, at 8 weeks, which is the usual age rodents are used for experiments. That would translate to a per-weight dose of ~250 µg/kg. Even if you used much older rats, who can weigh as much as twice as much, that would still translate to a dose of 125 µg/kg. So we're looking at a lipid nanoparticle does of ~18-35x higher (as a rough estimate) than the typical adult human dose. That's not unexpected. Biodistribution studies frequently use much higher doses than the human dose, the better to be able to detect distribution in low uptake organs, which, it turns out, the ovaries are.

As pointed out by multiple sources, the peak accumulation in the ovaries was 0.095% (or less than 1:1,000 of the total dose of lipid nanoparticle):

Yuri Deigin
@ydeigin · **Follow**

cite. No, it's not HUMAN ovaries, this was a RAT study! And less that 0.1% of the dose went there! 50% never even made it to tissues, 50% was at injection site, and then metabolized by liver. Brain peak was 0.02% (1/5000)

NurseErin @erin_bsn

There is evidence to suggest that the Covid mRNA-LNP (lipid nanoparticles) are adhering themselves to human organs (i.e. female ovaries). No long-term studies. You can never get unvaccinated. Leaked confidential study from Pfizer. files.catbox.moe/0vwcmj.pdf #knowledgeispower

5:25 PM · May 30, 2021

40 See the latest COVID-19 information on X

Read 32 replies

Not that this has deterred antivaxxers:

nametobe · May 29, 2021
@nametobe1 · **Follow**
Replying to @DFisman and @UofGuelphOAC
Not surprising that you are trying to discredit him. He doesn't support-vaccines are best- narrative.

Glen Pyle | #WomensHeartHealth
@glenpyle · **Follow**

The paper Byram cited doesn't support his claim. That's pretty telling that a study cited to support his claims actually goes against those claims.

9:51 PM · May 29, 2021

47 Reply Share

Read 9 replies

Because, of course, it hasn't.

At this point, I can't help but also point out that there have been actual studies of COVID-19 vaccines and ovarian function. In one such study, for example, researchers studied women undergoing oocyte retrieval for in vitro fertilization. They found no detrimental effect on ovarian follicular function. Another study of women undergoing in vitro fertilization demonstrated that the Moderna COVID-19 vaccine has no detectable effect on the percentage of clinical pregnancies resulting from the procedure. Yet another study has shown that vaccination against COVID-19 has no effect on immunological tolerance of the fetus by the mother. Still another study failed to find any effect on embryo implantation rates between SARS-CoV-2 infection seropositive, SARS-CoV-2 vaccine seropositive, or seronegative women.

It is true that most of these studies are still on preprint servers and haven't completed peer review yet to be published in a peer-reviewed journal, but the evidence thus far is *strongly* supportive of the conclusion that neither COVID-19 infection nor vaccination against COVID-19 has any effect on female fertility. While it is true that these studies all examine women undergoing in vitro fertilization procedures and the women who took part are thus not representative of all reproductive age women, these results are even worse for the claim that COVID-19 vaccines cause infertility. Why? Most of these women (surrogates and cases of male factor infertility excluded) undergo in vitro fertilization because of difficulty conceiving, and one would expect

such women to be *more*, not less, susceptible to anything that might impact female fertility. And, as was pointed out by others, several female participants got pregnant during Pfizer's phase 3 trial, and the only adverse pregnancy outcome was in the placebo group.

But what about the rest of the biodistribution?

As Yuri Deigin pointed out, only around 0.1% of the dose of lipid nanoparticles went to the ovaries. More importantly, 53% stayed at the injection site in the muscle, which is good, given that the whole point of an intramuscular injection is for the lipid nanoparticles to get the mRNA contained within into muscle cells near the injection cite, there to set up shop and provide the template for producing spike protein. Another "popular" site was the nearest lymph node basin, which explains why enlarged axillary lymph nodes (lymph nodes under the arm) have been observed in some women undergoing screening mammography too soon after vaccination, leading radiology and breast cancer specialists to tweak their mammography guidelines to minimize the chance of unnecessary axillary lymph node biopsies. At peak, only 0.02% made it to the brain.

Another thing about Dr. Bridle's statements that bothered me as I read them. He has conflated spike protein with lipid nanoparticles, not just once, but repeatedly. For example, in this article:

> "It's the first time ever scientists have been privy to seeing where these messenger RNA [mRNA] vaccines go after vaccination," Bridle explained. "Is it a safe assumption that it stays in the shoulder muscle? The short answer is: absolutely not. It's very disconcerting."
>
> Bridle argues that unlike traditional vaccines that stay mostly in the vaccination site at the shoulder muscle, the Japanese study showed how the spike protein of the coronavirus enters the bloodstream and circulates around the body for several days after a person gets inoculated with the vaccine.
>
> The spike protein then accumulates in organs and tissues such as the spleen, bone marrow, liver, adrenal glands, and in "quite high concentrations" in the ovaries.

No, the Japanese biodistribution study shows nothing of the sort. It only looked at where the lipid

containing an mRNA coding for Luciferase, a protein that exhibits bioluminescence allowing for visualization where the mRNA ended up. One interesting finding is that, after an intravenous injection, the predominant organ where the lipids in the lipid nanoparticles (ALC-0315 and ALC-0159) end up is the liver. Both lipids are eliminated rapidly from the plasma by several logs, such that by 300 hours (12.5 days) after injection very little is detectable. In the liver, however, the ALC-0315 takes around six weeks to be eliminated from the liver. One wonders, one does, why antivaxxers aren't going ballistic about this finding. Maybe it's because, at the peak, the amount of lipid nanoparticles detected in the liver was only 18%. Who knows? Maybe it's because saying that lipid nanoparticles cause female infertility is more scary than saying they take a while to be eliminated from the liver.

More tellingly, though, antivaxxers are portraying the Japanese biodistribution study as though it were some sort of "secret" document. ("Confidential"! Secret!) However, it has been public for some time. It's been publicly available for several months on the Japanese Pharmaceutical and Medical Devices Agency website, and the European Medicines Agency assessment report on the Pfizer vaccine repeatedly references results from the study.

The bottom line is that there is no evidence that the lipid nanoparticles in the Pfizer vaccine (or any of the COVID-19 vaccines) accumulate at significant quantities in the ovaries, much less cause female infertility. This new claim is nothing more than a repackaging of the previous claim that COVID-19 vaccines cause miscarriages and female infertility because of the supposed resemblance of sequences in the spike protein and the placental syncytin protein causing the immune response from the vaccine to attack syncytin, which was a repackaging of old antivaccine claims that vaccines sterilize women. Spike protein does not sufficiently resemble syncytin to cause miscarriages and infertility, and the lipid nanoparticles in the vaccines do not accumulate in the ovaries, much less cause female infertility.

In fact, this new version of the "vaccines are going to sterilize our womenfolk" disinformation leads me to conclude that just as aluminum became the new mercury as the science became clear that mercury in the thimerosal preservative that used to be in some childhood vaccines does not cause autism, lipid nanoparticles are the new polysorbate-80. Does anyone remember polysorbate-80 (also called Tween-80) or Triton X-100, detergents and emulsifiers used in some pharmaceutical products? Antivaxxers used to blame polysorbate-80 found in Gardasil for premature ovarian failure and female infertility. I first wrote about it nearly 13 years ago!

Everything old is indeed new again, with antivaxxers easily repurposing lipid nanoparticles into the role previously held by emulsifiers as the "culprits" in vaccines sterilizing women, all with a dollop of the "toxins" gambit. I can only speculate what farcical molecular "mechanism" antivaxxers will think of next to blame COVID-19 vaccines for sterilizing our womenfolk.

Author

- David Gorski

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This is Exhibit N referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

WORLD

Court intervenes after baby's parents refuse 'vaccinated blood' transfusion

By **Kathryn Mannie** • **Global News**
Posted December 7, 2022 11:52 am
4 min read

File - A view of the Auckland High Court. **Greg Bowker/Getty Images**

of a controversial **blood transfusion** case will be taken under the guardianship of health authorities so he can receive a life-saving operation.

His parents had been unwilling to proceed with the surgery over concerns he would receive "vaccinated blood," and were seeking a court order for their baby to receive blood from unvaccinated donors.

The parents said in previous interviews that the baby needed surgery "almost immediately," but they were "extremely concerned with the blood (the doctors) are going to use," the **Guardian** reported. The boy, referred to as Baby W, has a congenital heart defect and will not survive without an urgent operation.

The ruling to temporarily place the boy in the guardianship of his pediatric heart surgeon and cardiologist was met with fierce backlash from anti-vaccine protestors, who demonstrated outside the Auckland courthouse on Wednesday as the decision was handed down, the **New Zealand Herald** reported. The case has been a rallying point for the anti-vaccine movement in the country.

The presiding judge, Ian Gault, ruled that Baby W's temporary guardianship by health authorities will only last from Wednesday until he recovers from his life-saving surgery, which is expected to be by January 2023 at the latest. Gault emphasized that the boy's parents are still his primary guardians and doctors must keep them informed of his condition and treatment at all times. The parents retain guardianship of the child in all other matters.

heard arguments from Paul White, lawyer for Te Whatu Ora (Health New Zealand), Sue Grey, who represented the parents, and Adam Ross, a lawyer for the New Zealand Blood Service.

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White said that specialists believe the child's heart is suffering damage because of the surgical delays. Baby W is experiencing pulmonary valve stenosis, the narrowing of a heart valve, which is causing a build-up of blood and pressure, he said.

"His survival is actually dependent on the application being granted," White argued.

Meanwhile, Grey requested that the court order the country's blood service to establish a tailored donor service dealing in blood exclusively from unvaccinated people.

The New Zealand Blood Service argued that allowing parents to refuse vaccinated blood would set a dangerous precedent whereby patients could pick and choose where their donor blood comes from. Agency lawyer Ross said this would jeopardize the integrity of the blood service and lead to ethical and clinically bankrupt requests for blood.

Justice Gault ruled that the parents' request for unvaccinated blood was unnecessary and impractical, adding that the operation was in the child's "best interest" and there was "no scientific evidence" that vaccinated blood poses any risk, citing evidence provided by New Zealand's chief medical officer.

The judge also noted that the New Zealand Blood Service presented evidence from the past six months showing a "significant increase in potential blood recipients asking for blood from unvaccinated donors or asking about directed donation. Similar trends have been noted in other countries."

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During the hearing, the parents' lawyer cited an affidavit provided by a controversial Canadian academic, **Byram Bridle**, an associate professor at the Ontario Veterinary College at the University of Guelph. Bridle has been **publicly critical** of the safety of COVID-19 vaccines, for which he has faced criticism from the scientific and medical community.

Ross, the lawyer for the New Zealand Blood Service, took aim at Bridle's credentials, saying he was a doctor "of the PhD variety," not a medical doctor.

Bridle was recently chosen as an expert witness by a Toronto mother who was engaged in a court battle with her son's father over who should have the final say in their child's vaccinations. Bridle refused to acknowledge that the COVID-19 vaccine prevents serious illness and death, and the judge in the case ruled that he was not qualified to give an expert opinion.

"Respectfully, this is so far removed from the mainstream and widely accepted views of the Canadian and international medical and scientific community that the court cannot accept Dr. Bridle's evidence on the COVID vaccine as reliable," the judge ruled, as reported by **Guelph Today**.

"Dr. Bridle acknowledged that he is not a medical doctor. He has never vaccinated a child, he has never treated a child or an adult suffering from a reaction to a vaccine, nor has he ever treated a child or an adult who is suffering from an infectious disease," the ruling reads.

The judge ruled that the father in the Canadian case, who does not have custody of the child, was the best choice to make decisions about his son's vaccinations. The child's mother will retain custody.

1:50
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DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

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REPLY MOTION RECORD OF THE DEFENDANT DAVID FISMAN - Vol. 1 (Returnable November 19, 2024)

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ONTARIO
SUPERIOR COURT OF JUSTICE

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DR. BYRAM BRIDLE

Plaintiff

and

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Defendants

REPLY MOTION RECORD OF THE DEFENDANT, DAVID FISMAN - Vol. 2

(Returnable November 19, 2024)

March 15, 2023

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Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Science-Based Medicine
Exploring issues & controversies in science & medicine

Basic Science Neuroscience/Mental Health Vaccines

"New school" antivax goes old school as Byram Bridle asks if COVID-19 vaccines will drive an "epidemic" of autism

Wakefield redux? Antivax scientist Byram Bridle just took the "new school" antivax movement old school by implying that COVID-19 vaccines might cause an "epidemic of autism." Everything old is new again, sort of.

David Gorski on January 22, 2024

Post

Sometimes there are topics that just demand that I write about them. So it was over the weekend, as I was perusing my usual sources looking for suitable topics to write about today and found one that grabbed my attention to the point where I immediately knew that I had to abandon the topic that I had already tentatively chosen for today, putting it off for another day (or perhaps my not-so-secret other blog) and focusing on this. I will freely admit that part of the reason I knew I had to write about this was because it so clearly vindicated what for me had started out as a bit of a quip that I had started repeating in the early months after the mRNA-based COVID-19 vaccines from Pfizer and Moderna first rolled out in those heady days of early 2021, namely that the only reason antivaxxers weren't claiming that COVID-19 vaccines could cause autism was because they were not being administered to young children. As the age range for the COVID-19 vaccines got younger and younger, I predicted that antivaxxers would soon be blaming them for autism, but that it might take a few years because autism spectrum disorders are usually not diagnosed until a child is around 3 years old and the characteristic behaviors start to manifest themselves to the point where the parents become concerned. Leave it to Byram Bridle to vindicate my prediction with a post over the weekend on his Substack entitled Will COVID-19 Shots Drive an Epidemic of Autism?, albeit not in the way that I expected. He did, however, echo a common variant of an old antivax claim that vaccines cause autism by adding the blurb *If Yes, Those Who Coerced Pregnant Women to Take modRNA Shots Will Be Responsible For It*.

It all very much reminded me of something I said on X, the platform formerly known as Twitter, the other day:

Byram Bridle returns! (He never really went away)

You might remember Dr. Byram Bridle, associate professor and viral immunologist in the Department of Pathobiology in the Ontario Veterinary College at the University of Guelph, from the early days of the COVID-19 vaccine rollout nearly three years ago. I first wrote about him in June 2021, when he claimed to have "discovered" a "secret" Japanese study that showed that the Pfizer vaccine's biodistribution included the ovaries (among other organs), ignoring the facts that the study was not "secret" and was a fairly standard biodistribution study in which huge doses of the vaccine were injected intravenously in mice in order to determine where the drug might go, no matter how small the quantities. (Remember, vaccines are not injected directly into the bloodstream, much less at doses anywhere near what the study used.)

Basically, Dr. Bridle (mis)represented himself as a "provaccine" scientist who made this startling admission (tellingly, quoted by Mike Adams):

> "In short, the conclusion is ***we made a big mistake***. We didn't realize it until now. We saw the spike protein was a great target antigen. We never

> So, by vaccinating people, we are inadvertently inoculating them with a toxin, and in some people this gets into circulation. And when that happens in some people, it can cause damage, especially with the cardiovascular system. I don't have time, but many other legitimate questions about the long-term safety there for this vaccine. For example, with accumulating in the ovaries, one of my questions is, "will we be rendering young people infertile, some of them infertile?" So, I'll stop there.

Yep, it's the "vaccines are going to sterilize our womenfolk" gambit all over again, an antivax gambit that started right around the time that COVID-19 vaccines were granted emergency use authorization (EUA) in December 2020. It's a very old antivax trope that dates back decades, which is why I was not surprised to see it rear its ugly head again applied to the new mRNA-based vaccines. How old is it? Well, as a matter of coincidence, a peer-reviewed article co-authored by Tara Smith and me accepted for publication in *Vaccine* just hit the journal's website late last week. (That's the first good thing that's happened to me in 2024.) In the article, we discuss how this trope goes back at least to the 1990s and why it's a nonsensical conspiracy theory.

But back to Dr. Bridle, given that this time he is not fear mongering about COVID-19 vaccines "sterilizing the womenfolk." During the last three years, he has diversified his antivax propaganda; for instance, by sarcastically fear mongering about mRNA in breast milk, credulously accusing "Them" of "suppressing" data supposedly showing that ivermectin works against COVID-19 (it doesn't), parroting the antivax line about "adulteration" of mRNA-based vaccines with plasmid DNA (which is a big nothingburger), and showing up at all sorts of conspiracy theorist antivax conferences and panels. Basically, as much as he claims to be "not antivax," by his words and actions Dr. Bridle has demonstrated himself to be very much antivax indeed, right up to amusingly whining about how provaccine scientists (like Timothy Caulfield) and advocates "won't debate him."

COVID-19 vaccines and autism?

Based on his history, it should come a surprise to no one that Dr. Bridle finds a study done in rats very persuasive support for the contention that prenatal exposure to COVID-19 vaccines will cause autism. It should also come as no surprise given the long history (some of which I myself have documented here) of antivax scientists doing dubious studies of vaccines or vaccine ingredients, like the mercury-containing preservative thimerosal back in the day when thimerosal-containing vaccines were demonized as The One

True vaccine cause of autism, at least here in the US. Dr. Bridle's post also reminded me of a number studies cited by antivaxxers to claim that "immune activation" due to prenatal exposure to, say, Tdap vaccines during pregnancy can hugely increase the risk of autism in the child after birth. (They don't.) So, right off the bat, we know that Dr. Bridle is resurrecting old antivax misinformation and just applying it to COVID-19 vaccines.

Let's look at his "concerns" about the study before I discuss the study itself, to see how the study is being spun:

> "I just finished reading a peer-reviewed scientific article that has been electronically published ahead of the print version. The findings have caused me considerable concern. The paper is entitled "Prenatal Exposure to COVID-19 mRNA Vaccine BNT162b2 Induces Autism-Like Behaviors in Male Neonatal Rats: Insights into WNT and BDNF Signaling Perturbations".

Of course, the findings caused Dr. Bridle "considerable concern." He's antivax, which leads him to this statement:

> "Sadly, we should have had this comprehensive understanding and then conducted a fully informed risk-benefit analysis **before** hundreds of thousands of pregnant women were inoculated. But, maybe that is just my crazy way of thinking.

Funny how antivaxxers will seize upon a single study in rats as reason for incredible concern and ignore the clinical data that we have from huge numbers of people vaccinated during pregnancy whose sum total has failed to find evidence adverse maternal or fetal effects from vaccinating pregnant individuals with the COVID-19 vaccine but has produced a growing body of data demonstrates the safety of such use. (There's even data that the vaccine administered during pregnancy might provide some immunity to the baby as well.) Yeah, a single rat study is going to overturn all that clinical data, because…reasons.

Yes, Dr. Bridle airily dismisses all the existing evidence thusly:

> "The published studies looking at this have largely been flawed and biased. Ever-emerging evidence proves that the harms of the shots have

> been substantially underestimated in all people. The only question is by how much? Worse, the studies done during pregnancy have focused most heavily on the women, and less so on the babies. In many cases, babies were followed for only a hand-full of weeks following their birth. This is much too short. And evaluations of these babies have been overly superficial. A classic trick when it comes to the research and development of novel medical interventions is this: one can make a product appear quite safe if one does not look very hard for harms.

Got that? That's standard antivax conspiracy patter claiming that the only reason "They" don't find vaccine harms is because "They" don't look very hard. I swear, I can't even remember the first time I heard that antivax trope because it was so long ago, nor can I remember the number of times I've heard the conspiracy theory that "They" are "suppressing evidence" of harms that antivaxxers so desperately want to attribute to vaccines.

Let's look at the study now.

COVID-19 vaccines and autism…in rats?

The study in question was published a couple of weeks ago in a journal that I've never heard of before, *Neurochemical Research*, and entitled Prenatal Exposure to COVID-19 mRNA Vaccine BNT162b2 Induces Autism-Like Behaviors in Male Neonatal Rats: Insights into WNT and BDNF Signaling Perturbations. The journal itself, I found, has a respectable impact factor for a specialty journal (4.4.14 in 2023-4); so it's not the typical crap journal that I usually see these sorts of studies in. It's also garnered an Altmetric score of 2,757 as of yesterday, which is very high, in the 99th percentile, and has been accessed 44K times. As for the investigators, I am not familiar with any of them, but they are Turkish and come from Izmir Katip Celebi University, Istinye University, Afyon Kocatepe University, and Demiroğlu Bilim University, and the first (and corresponding author) is someone named Mumin Alper Erdogan, who last October also published a study in *Journal of Neuroimmune Pharmacology* entitled Prenatal SARS-CoV-2 Spike Protein Exposure Induces Autism-Like Neurobehavioral Changes in Male Neonatal Rats.

This latter study is different in that Erdogan injected pregnant rats with either saline, large amount of aluminum hydroxide adjuvant (150 µg/kg, which is within the range commonly recommended for preclinical rodent models), or a large amounts of spike protein (40 µg/kg!) plus a large amount of aluminum adjuvant and then tested the litters of mice for various behavioral traits used as models of autistic behavior, as well as immune markers thought to be associated with autism. Again, I was struck at how large the dose of

spike protein was, microgram quantities in rodents that only weighed around 220 g, compared to the typical 70,000 g adult male human. This study was sold as indicating that COVID-19 infection during pregnancy could increase the risk of autism in the offspring of the pregnancy, finding:

> At P50, we conducted behavioral analyses on these mature animals and performed MR spectroscopy. Subsequently, all animals were sacrificed, and their brains were subject to biochemical and histological analysis. Our findings indicate that male rats exposed to the spike protein displayed a higher rate of impaired performance on behavioral studies, including the three-chamber social test, passive avoidance learning analysis, open field test, rotarod test, and novelty-induced cultivation behavior, indicative of autistic symptoms. Exposure to the spike protein (male) induced gliosis and neuronal cell death in the CA1-CA3 regions of the hippocampus and cerebellum. The spike protein-exposed male rats exhibited significantly greater levels of malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α), interleukin-17 (IL-17), nuclear factor kappa B (NF-κB), and lactate and lower levels of brain-derived neurotrophic factor (BDNF) than the control group. Our study suggests a potential association between prenatal exposure to COVID-19 spike protein and neurodevelopmental problems, such as ASD. These findings highlight the importance of further research into the potential effects of the COVID-19 virus on embryonic and fetal development and the potential long-term consequences for neurodevelopment.

My first question, of course, was why there was no spike protein alone group, because such a group would help figure out whether the immune system needed to be more stimulated, as with an adjuvant, for this effect to be observed. Still, my eyebrow was raised when I read this passage:

> In light of the growing body of research indicating a connection between SARS-CoV-2 infection and neurological symptoms, we conducted a study to examine the potential impact of a synthetic version of the SARS-CoV-2 spike protein on the development of autism spectrum disorder (ASD) in offspring born to mothers exposed to the protein

during pregnancy.

And, later:

> In light of the growing body of research indicating a connection between SARS-CoV-2 infection and neurological symptoms, we conducted a study to examine the potential impact of a synthetic version of the SARS-CoV-2 spike protein on the development of autism spectrum disorder (ASD) in offspring born to mothers exposed to the protein during pregnancy.

Why the synthetic version? Why not compare to viral infection itself? Why such huge doses? Maybe I'm overly suspicious, but I get the feeling that this study was done in order to lay the groundwork for doing the study being promoted by Dr. Bridle that purports to show a link between the vaccine and autism after prenatal exposure to the vaccine. However, I'll just take the Erdogan and the authors at their word for the moment that their intent was to study the neuroinflammatory effects of spike protein from infection as a potential promoter of neurodevelopmental disorders like autism spectrum disorders. The reason? Motivation doesn't matter. Results do. Also, relevance matters, and these studies, as you will see, are not particularly relevant to the questions to which Dr. Bridle thinks they are very relevant.

Let's move on to the study that so "concerns" Dr. Bridle, who opines later in his post:

> I have two boys, so I became very familiar with babies a bunch of years ago. Let's face it, the bulk of a baby's early life consists of crying, drinking/eating, pooping/peeing, and sleeping. There is simply not a lot to evaluate when it comes to harms to babies that would manifest as behavioural issues. It is not unusual for babies to seem unhappy and uncomfortable on a regular basis. In other words, one cannot be confident in declaring an absence of a disorder in newborns and even toddlers. For example, I have heard that failing to make eye contact can be a potential sign of autism in young children. Obviously, such an observation cannot be made in a baby; they don't start making consistent and intentional eye contact for quite some time. So, be very careful when you hear people declaring that there is no evidence of harm

> to children born to mothers that received the COVID-19 shots. There simply has not been enough time to properly evaluate this.

I like to point out that signs of ASD are often detected in children less than two and can be detected in infants as young as age 6-12 months, sometimes even as young as 3 months. When it comes to ASDs, Dr. Bridle simply doesn't know what he is talking about here. There is even evidence that signs of autism can be detected in utero as early as mid-gestation. A little humility is in order here. For instance, I know that I am not an expert in neuroscience, autism, or neurodevelopment. I do, however, know from my many years combatting antivaccine misinformation claiming that vaccines cause autism that the signs of autism are detectable much earlier than antivaxxers commonly argue.

Not that that stops Dr. Bridle:

> "If COVID-19 shots administered to pregnant women can cause neurological harms in their developing babies, including alterations that lead to things like autism, it is almost certain that these could not be identified within the first six weeks. Humans need to gain certain abilities to interact with the world around them before health care professionals can properly assess for evidence of complex disorders. My goodness, some harms aren't noticed until adolescence if hormonal changes are required to unveil them. This means there is the potential for harms to babies born to mothers that were coerced into getting the COVID-19 shots that we are still unaware of.

No one ever said that we could evaluate in a mere six weeks whether COVID-19 vaccines affect pregnancy outcomes. Again, though, he doesn't know what he's talking about here. You don't have to wait until adolescence to see the signs of ASDs! Again, in some cases, the signs of ASDs can be detected in infancy. His entire argument is just an excuse to invoke the "no long term safety studies" gambit beloved by antivaxxers.

Let's get to the study, though.

It's a fairly straightforward study design, although not necessarily a straightforward study. Erdogan's justification is:

> "While the mRNA vaccines have exhibited exceptional efficacy in

> forestalling severe COVID-19 manifestations and curtailing viral transmission, it is of paramount importance to scrupulously investigate the potential neurological ramifications associated with the spike protein itself and with the immune response it induces [16, 17]. Gaining a comprehensive understanding of the implications of spike protein-induced neuroinflammation, along with its impact on synaptic plasticity and overall brain development, will bolster our knowledge of the long-term effects of COVID-19 infection and its vaccination [18].
>
> In this study, our primary objective is to delve deeply into the existing literature and data concerning the potential relationship between COVID-19 mRNA vaccines, spike protein-mediated reactions, and the genesis of neurodevelopmental disorders, focusing on autism.

And:

> Through a detailed analysis of these carefully selected parameters, we aim to provide a clearer picture of this pressing area of research and offer directions for subsequent investigations [19, 20].

Is it that pressing a direction of research, though? Is it really? Current clinical evidence in hundreds of thousands, if not millions, of pregnant humans and their offspring have not raised a safety signal of neurodevelopmental disorders linked to the vaccine.

Dr. Bridle cites a reference indicating that many cases of ASD are not diagnosed before the age of 3 and some are not diagnosed until age 5, which is true enough, but not entirely relevant, not that that stops Dr. Bridle:

> Here is the problem: Children born to mothers that were coerced into taking COVID-19 shots have not yet reached the age of three. We must now wait another 1.5 years, at least, and likely two or more years to accrue data from enough children to determine whether harms have been caused.

getting quite close to 3 years of age, and, again, there are even more who are two years old and still more who are 12 months old. There's nothing magic about being three years old. It's more like an average. Lots of children are diagnosed earlier than that, and lots are diagnosed later than that. Although it is still early, with COVID-19 vaccines having only been authorized during pregnancy less than three years ago US, it's not so early that safety signals for neurodevelopmental disorders in children born after having been exposed to the vaccine *in utero* through vaccination during pregnancy would not be starting to appear. It's also a shame, because Erdogan has also published some interesting work in areas in which I'm interested, such as the role of ion channels in cancer. (I wonder if I met him in London in 2015, when I gave a talk at a conference on that topic.)

So how was the study carried out? In brief:

> "Two cohorts of female rats were randomly assigned to the following treatment groups: Group 1 or the 0.9% NaCl Saline Group (n = 7) and Group 2 or the COVID-19 m-RNA Vaccine BNT162b2 Group (n = 8). Throughout the experiment, the behavior and physical well-being of all animals were meticulously monitored daily. To facilitate the mating process, three female rats were cohabitated with a single male rat for a period spanning two to three days during the estrus phase. The presence of white vaginal plaque in the female rats was used as an indicator of successful mating. After this occurred, the male rats were removed from the enclosures.

After the mating, the rats were then treated thusly:

> "Rats belonging to Group 1 were administered 1 ml/kg of 0.9% NaCl saline intramuscularly on the thirteenth day of gestation. Simultaneously, rats in Group 2 received a dosage of 30 µg/Rat of the COVID-19 m-RNA Vaccine BNT162b2 intramuscularly on the same day of pregnancy.

Let me first note that this is the ***full*** human dose of the Pfizer vaccine administered to rats whose average weight was only 220 ± 10 g. I realize that most people outside of the US are familiar with metric, but for nonscientists understand in the US I'll just say that this is the equivalent of ~7.8 oz, less than half a pound.

> Pregnant rats received the same dose as what is given to pregnant women. Some will try to argue that this represents, on a per body weight basis, a higher effective dose. It might, but we don't have proof of this. How drugs work can vary across species. There are examples where tiny rodents like mice could tolerate doses of drugs that were toxic in people.

Of course, that argument goes both ways, too. Many are the effects seen in rodent models due to drugs or other toxins that do not translate to people, although that doesn't stop Dr. Bridle from arguing:

> This research was conducted in rats. Pre-clinical studies like these are used to predict what might happen in humans. Sometimes the predictions are close. Sometimes the phenomena do not translate into people at all. And sometimes things are worse in people.

He also tries to hand wave why things might be worse in people:

> The authors assume that much of the toxicity to the rats is mediated by the spike protein that the modRNA shots get cells in the body to manufacture. However, what they fail to acknowledge is that rats express the low-affinity receptor for the spike protein, unlike humans that express the high-affinity receptor. This means that the spike can only bind weakly to rat cells, but strongly to human cells. As such, I suspect that most of the harm observed in the rats might have been due to inflammation mediated by the lipid nanoparticles and/or immune responses against the spike proteins. If the spike proteins could contribute to the toxicity, then matters could be much worse in hosts with cells expressing high-affinity receptors, which would include people.

This is what we in the biz like to call JAQing off, "just asking questions" designed to lead the listener towards, in this case, the antivax viewpoint being promoted on Dr. Bridle's Substack posts that the COVID-

of pregnancy status, gender, race, age, etc.).

To continue in that theme, Dr. Bridle argues:

> “ Further, the rats in this study were given a single dose, whereas pregnant women can receive up to two doses. The only way to know for sure is, unfortunately, to wait and see how the global human experiment pans out.

We’re already seeing that. The vaccines are safe and effective, albeit, thanks to mutation and the arrival of the Delta, Omicron, etc. variants and their ability to escape immunity from infection or vaccination against prior variants, they are less effective than they were three years ago.

Getting back to the study again, I note that there was no significant difference between the number of offspring per litter and no neonatal deaths among the litters in either group. Beginning on day 50 after birth (reset to day 1 for purposes of when the tests were administered), the rats born to Group 1 (controls) and Group 2 (vaccinated) were subjected to a number of behavioral tests:

> “ Open Field Test: This initial assessment took place on Day 1. Serving as both a measure of general locomotor activity and anxiety, it also acclimated the rats to a testing environment. Novelty-Induced Rearing Behavior: On Day 4, the rats were evaluated for their vertical explorative behaviors in a novel setting. Three-chamber Sociability and Social Novelty Test: Administered on Day 7, this assessment provided insights into the rats’ sociability and their preference for social novelty. Their prior acclimation to the testing conditions by this point ensured accurate insights. Rotarod Test: On Day 10, the rats underwent this test to evaluate their motor skills and endurance. This physically demanding test was placed last in the sequence to minimize any fatigue or stress impacts on the outcomes of the earlier behavioral assessments.

One thing I noticed right away is something I frequently notice basic scientists failing to do when evaluating the results of rodent experiments, namely doing their observations under double-blind conditions, such that the evaluators do not know which group each rat being tested comes from. They state that they used an

anywhere yesterday. One can argue that some of the tests, such as the three-chamber test in which the rat decides between a chamber with no other rat and a “stranger” rat or between a chamber with a known rat and a “stranger” rat in which the test subject’s inclinations to be with one or the other are measured, don’t necessarily need to be double-blinded, but I will argue that all immunohistochemistry tests need to be double blind, even if a computer-aided counting system is used. The reason is that there is some subjectivity in all immunohistochemistry, and, in this case:

> Systematic random sampling was employed to maintain uniformity across sections and minimize sampling bias. Counting was facilitated by an image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc., USA) to ensure accuracy and consistency. To ensure objectivity, neuronal counts were independently verified by two trained observers, with any discrepancies discussed and resolved to achieve a consensus count.

See why I think double-blinding would have been nice here?

In addition, several cytokines and immune system factors were measured by PCR from tissue harvested from the hippocampus of the rat brains.

In any event, key findings of the study included:

- Differences in WNT gene expression and brain-derived neurotrophic factor (BDNF) in vaccinated rats, both male and female.
- A substantial decrease in neuronal counts in critical brain regions, indicating potential neurodegeneration or altered neurodevelopment
- Male rats reportedly demonstrated “pronounced autism-like behaviors, characterized by a marked reduction in social interaction and repetitive patterns of behavior.”
- Male rats also demonstrated impaired motor performance, evidenced by reduced coordination and agility.

Here’s Table 4, showing some of the difference:

Sex	Male Groups	Female Groups

Groups	**% 0.9 NaCl Saline Male Group**	**COVID-19 m-RNA Vaccine BNT162b2 Male Group**	**% 0.9 NaCl Saline Female Group**	**COVID-19 m-RNA Vaccine BNT162b2 Female Group**
Brain IL-17 level (pg/mg protein)	109.7 ± 6.9	112.09 ± 3.8	128.6 ± 4.5	118.9 ± 3.8
Brain BDNF level (pg/mg protein)	806.8 ± 33.4	680.7 ± 27.6 #	773.6 ± 25.4	628.9 ± 24.9 *
Brain TNF-alpha level (pg/mg protein)	94.6 ± 0.3	92.09 ± 4.4	111.3 ± 4.1	104.3 ± 4.5
Brain IL-1 level (pg/mg protein)	22.7 ± 1.01	19.6 ± 0.5	22.7 ± 0.4	21.9 ± 0.9

1. Results were presented as mean ± SEM. Statistical analyses were performed by one-way ANOVA. # $p < 0.001$, different from % 0.9 NaCl Saline Male Group and * $p < 0.001$, different from % 0.9 NaCl Saline Female Group. For male rats, a significant decrease in brain BDNF levels was evident in the vaccinated group ($F(1, 36) = 10.24$, $p < 0.001$), suggesting a specific impact of the vaccine on neurotrophic factors. Similarly, for female rats, brain BDNF levels were significantly lower in the vaccinated group as compared to controls ($F(1, 36) = 11.67$, $p < 0.001$), reinforcing the potential influence of the vaccine on neurotrophic signaling

I am familiar with the role of WNT signaling (a molecular pathway that transmits extracellular signals to the nucleus to turn on and off sets of genes that modulate its effect) in cancer, but less so in the brain. The simple version is that WNT signaling is important in neurodevelopment and that impaired WNT signaling can result in various neural impairments, and some research implicates it in the development of ASDs. As

for BDNF, it too is important in neurodevelopment, and there is research implicating abnormalities in BDNF signaling with ASDs.

Also, there was noted to be a difference in neuron counts in the hippocampus:

Sex	Male Groups		Female Groups	
Groups	% 0.9 NaCl Saline Male Group	COVID-19 m-RNA Vaccine BNT162b2 Male Group	% 0.9 NaCl Saline Female Group	COVID-19 m-RNA Vaccine BNT162b2 Female Group
Neuronal Count CA1	71.8 ± 3.5	54.5 ± 1.3 #	68.5 ± 1.9	66.1 ± 0.8
Neuronal Count CA3	43.6 ± 1.7	30.2 ± 1.1 #	42.2 ± 1.3	39.1 ± 1.1
Purkinje Count Cerebellum	21.9 ± 1.4	13.5 ± 0.9 #	19.8 ± 0.6	18.3 ± 0.9

1. Results were presented as mean ± SEM. Statistical analyses were performed by one-way ANOVA. # $p < 0.001$, different from % 0.9 NaCl Saline Male Group. For the male groups, significant decreases in neuronal counts were observed in the CA1 ($F(1, 36) = 23.45$, $p < 0.001$), CA3 ($F(1, 36) = 28.37$, $p < 0.001$), and Purkinje cell count in the cerebellum ($F(1, 36) = 34.12$, $p < 0.001$) regions, indicating a robust effect of the vaccine. Conversely, the female groups did not show significant differences between treated and control groups in the CA1 ($F(1, 36) = 3.21$, $p = 0.081$), CA3 ($F(1, 36) = 4.78$, $p = 0.034$), and Purkinje cell count in the cerebellum ($F(1, 36) = 5.52$, $p = 0.024$)

Let's for a moment just accept these results as accurate, the way Dr. Bridle did. Let's say that the findings of differences in cytokine profiles and neuronal counts between the offspring of vaccinated and unvaccinated rats, as well as the behavioral differences observed, were accurately measured. What does this mean?

Before I answer that question, I can't help but mention that I'm glad actually perused the references cited in the study and came across this reference, buried among multiple citations to support a claim:

45. Seneff S, Nigh G, Kyriakopoulos AM, McCullough PA (2022) Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs. Food Chem Toxicol 164:113008

Article CAS PubMed PubMed Central Google Scholar

Erdogan cited Stephanie Seneff and Peter McCullough? Uh-oh. Maybe I was too kind…

Yikes. And uh-oh…again:

47. Kyriakopoulos AM, McCullough PA, Nigh G, Seneff S (2022) Potential mechanisms for human genome integration of genetic code from SARS-CoV-2 mRNA vaccination: implications for disease. J Neurol Disord 10:519

Google Scholar

No wonder Dr. Bridle likes this study. The authors have cited two of the worst antivax papers I've ever seen about COVID-19 vaccines whose authors include one of the worst "new school" antivaxxers (McCullough) and one of the worst "old school" antivaxxers (Seneff).

I take back what I said above when I questioned whether I was being too suspicious of this paper. When you cite two Stephanie Seneff papers, you deserve the suspicion that I had, and it isn't surprising to me that Dr. Bridle likes this paper. If anything, I was probably not suspicious enough about Erdogan, particularly given the conclusion made by him and his coauthors:

> "Given the public health significance of understanding the effects of COVID-19 vaccination, especially during pregnancy, comprehensive studies are vital. These should weigh the benefits and potential risks of vaccination, focusing on ensuring optimal neurodevelopmental outcomes. Our findings underscore the importance of continued research in this domain to guarantee the safety and well-being of all

> In summary, this study provides valuable insights into the effects of the COVID-19 mRNA BNT162b2 vaccine on the WNT pathway and BDNF levels, particularly in relation to neurodevelopmental outcomes. The observed male-specific vulnerability and the convergence with existing literature support the involvement of these molecular pathways in neurodevelopmental disorders. However, further research is warranted to validate these findings in human populations and to unravel the complex mechanisms underlying the observed effects. The ultimate goal is to ensure the safety and well-being of individuals receiving COVID-19 vaccination, particularly during pregnancy, while minimizing potential risks to neurodevelopment.

More of a favorite antivax gambit, namely "we need more research." It's a gambit used no matter how massive the evidence base against an antivax claim is, such as the old claim that vaccines cause autism.

I deconstructed in depth that paper on G-quadruplexes nearly two years ago! As for the paper on "human genome integration," I didn't discuss that paper in particular, but did discuss many times how the claims that the mRNA vaccine can "integrate" into the genome is nonsense. No wonder Dr. Bridle and other antivaxxers like this study!

Relevance, ignored by antivaxxers like Dr. Bridle

The question one has to ask when confronted with a study like this is one of relevance to humans. Even Dr. Bridle recognizes this issue, as he spends considerable verbiage trying to dismiss criticisms based on whether this study is even relevant to what happens in humans while JAQing off in order to make you think that we must treat the results as relevant even though there are a lot of reasons not to, as well as a large amount of data from epidemiological studies of the use of COVID-19 vaccines during pregnancy.

Enter the pandemic's wrongest man, Alex Berenson, echoing Dr. Bridle's nonsense about this study:

giving mRNA COVID jabs to pregnant rats damages the brains of their offspring

URGENT: Giving mRNA Covid vaccines to pregnant rats caused brain changes and autism-like behavior in their young, a new study shows

Naturally, the disturbing finding - which was published in a respected, peer-reviewed journal last week - didn't come from American scientists.

ALEX BERENSON

JAN 19, 2024

2:05 PM · Jan 19, 2024 · **84K** Views

@AlexBerenson

Article here:

alexberenson.substack.com/p/urgent-givin...

2:13 PM · Jan 19, 2024 · **14.8K** Views

5 9 41 4

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Reply

Alex Berenson @AlexBerenson · Jan 19

Original paper here:

Prenatal Exposure to COVID-19 mRNA Vaccine BNT162b2 Induces Autism-Like...

From link.springer.com

7 16 56 15K

Naturally, like Dr. Bridle, Mr. Berenson trumpeted this on his Substack in an article entitled URGENT: Giving mRNA Covid vaccines to pregnant rats caused brain changes and autism-like behavior in their young, a new study shows, with the conspiratorial tagline: *Naturally, the disturbing finding – which was published in a respected, peer-reviewed journal last week – didn't come from American scientists* and a sarcastic

> *(Luckily Pfizer did a ton of work to make sure this wouldn't be problem in humans. Oh, they didn't? Hey, everyone makes mistakes.)*

Again, it's all about the fear and doubt about the vaccines and ignoring all the human evidence out there:

> The study coved only about 40 rats, and it does not prove the vaccines cause autism or similar brain changes in the children of vaccinated pregnant women.
>
> But it does show – again – that the jabs can cause powerful inflammatory and autoimmune responses with unknown consequences, and that their long-term risks have barely been studied.

Mr. Berenson is also ready with an excuse that antivaxxers have been making for two decades:

> But neither governments nor vaccine companies have shown any inclination to do that work. And so even if autism diagnoses notably rise in the next few years, proving the mRNAs are responsible will be next to impossible.

Actually, it would be quite possible, in the very same way that doctors studied the vaccine schedule and tried to correlate it with autism diagnoses for the last 30 years and have failed to find a link. It's just that antivaxxers won't accept those results. They didn't accept them for the childhood vaccine schedule, and they won't accept them for COVID-19 vaccines.

Again, Mr. Berenson is JAQing off, just like Dr. Bridle. Again, it is very much unclear how relevant a model can be in which a dose of vaccine relative to animal body weight so much larger than what is administered to humans and the physiology of pregnancy is so different. One can also question how relevant the behavioral models are to human autism. For example, the three-chamber test has a definite drawback:

> To test a mouse's social interest, researchers place it in a cage that has two side chambers, one of which holds another mouse. Mice normally prefer to investigate the mouse rather than spend time in the empty

> space. Some of the autism mouse models spend as much or more time in the empty cage, which researchers take to be an indication of social deficits.
>
> But this test relies on an artificial scenario, in which the new mouse is caged and cannot initiate social contact, says Paylor. Tellingly, the FMR1 and NLGN3 rat models both ignore social play, but act normally in the three-chamber test.
>
> "Having a barrier between animals may completely alter the way animals normally interact, and certainly in young rats it prevents rough-and-tumble play behavior," says Paylor.

I also note that the rats in the Erdogan study were not pups. They were 50 days old and had reached maturity, which is rather late to have been studying the rats. Indeed, one could question why the behavioral part of the study was even designed the way it was when there are rat models that can examine behaviors more relevant to ASDs. I get it. The three-chamber model used is fairly simple and straightforward, but it's not particularly relevant, particularly not with mature rats.

I could go on and on and on, but I think one of the most telling things I noticed was the reappearance in the comments of Mr. Berenson's post of an old "friend," J.B. Handley, founder of Generation Rescue and old school "mercury in vaccines causes autism" leader who's been fairly quiet for quite a few years. Amusingly, he's annoyed at Mr. Berenson for previously not having been willing to consider this:

> "Alex: Your unwillingness, to date, to consider the childhood vaccine schedule's role in the autism epidemic has been very frustrating for people like me who otherwise conisder you a hero. I hope this revalation helps you re-consider that somehow, magically, the childhood vaccines cause no problems…If you are looking for a mechanism of action, Cal Tech's Paul Patterson proposed a very clear one more than a decade ago: immune activation events in the brains of babies during critical periods of brain formation. These simmering brain infections can be caused my many different antagonists, but man-made nanoparticle aluminum (the kind found in vaccines that isn't found in nature) is an obvious culprit, because lymphocytes transport it to the brain and the body can't excrete

> it. There's likely something similar happening with the mRNA shots that hit the brains of the baby rats, perhaps it's the spike protein.

I was amused. Mr. Berenson is not sufficiently antivax for old-school antivaxxers, although apparently Dr. Bridle is.

As I wrote at the beginning this post, there is nothing new under the antivax sun. Every antivax claim made about COVID-19 vaccines has a precursor from the beforetime. Also, as I said above, it is nearly inevitable that "new school" antivaxxers who became antivax because of COVID-19 vaccines will over time become become just antivax. As it becomes clear that there is no increased risk of neurodevelopmental disorders like ASDs in children born to individuals vaccinated during pregnancy, the focus will shift (is likely already shifting) to blaming COVID-19 vaccines given to 6 month olds for autism. Same as it ever was.

Author

- David Gorski

Dr. Gorski's full information can be found here, along with information for patients. **David H. Gorski, MD, PhD, FACS** is a surgical oncologist at the Barbara Ann Karmanos Cancer Institute specializing in breast cancer surgery, where he also serves as the American College of Surgeons Committee on Cancer Liaison Physician as well as an Associate Professor of Surgery and member of the faculty of the Graduate Program in Cancer Biology at Wayne State University. If you are a potential patient and found this page through a Google search, please check out Dr. Gorski's biographical information, disclaimers regarding his writings, and notice to patients here.

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This is Exhibit P referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Laura Ingraham guest pushes debunked claims that the COVID-19 vaccines are a "toxin"

Dr. Byram Bridle has cited studies whose original authors say he is "completely misinterpreting" their research

WRITTEN BY ERIC KLEEFELD

PUBLISHED 08/04/21 11:23 AM EDT

Fox News host Laura Ingraham continued her show's running campaign against the COVID-19 vaccines on Tuesday night, bringing on a guest to fearmonger that the vaccines are a "toxin" and dangerous to people who take them. In fact, experts have countered for the past two months that his arguments are misleading, and he is cherry-picking data from studies that show the vaccines actually working properly.

Ingraham introduced Dr. Byram Bridle, an associate professor of viral immunology at the University of Guelph's Ontario Veterinary College in Canada, by claiming he found that "the spike protein produced by the mRNA vaccines does not remain in the shoulder muscle upon injection, but rather gets into the blood — and can, in some cases, lead to clotting, bleeding, heart problems, neurological damage, and more."

Ingraham then played a clip of Bridle from May 27, in which he said: "We never knew the spike protein was a toxin and was a pathogenic protein. Was it accumulating in the ovaries, one of my questions is, will we be rendering young people infertile, some of them infertile?"

But a key thing to understand here is that Bridle has not conducted his own studies, but has reviewed papers from other people — and those same people say that he's presenting their work completely wrong.

From the August 3, 2021, edition of Fox News' *The Ingraham Angle*

THE LATEST

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IN THIS ARTICLE

Coronavirus (COVID-19)

Laura Ingraham

Fox News

key details of a paper that found small amounts of the vaccine-generated spike proteins in the bloodstreams of 13 health care workers.

"The spike became undetectable by 14 days after the first dose of the vaccine," Matchett said, explaining that the study used a highly sensitive detection method. "After the second dose, they could not detect the spike protein in the blood of any of the participants because the participants had all generated anti-spike antibodies."

Reuters also explained: "This is because the individuals developed antibodies to remove the antigen from the bloodstream, creating an immune response exactly as the vaccine was designed to do."

Another study co-author, David Walt of Harvard Medical School, told Reuters, that "Bridle is taking our results and completely misinterpreting them." He added: "The most important message is over 400 million doses of the mRNA vaccine have been administered with negligible serious consequences. It is incredibly safe."

Jason McLellan, a structural biologist at the University of Texas at Austin, explained to FactCheck.org that the spike proteins encoded by the vaccines have a key difference from the normal spike proteins in the virus, which prevents them being able to change shape into their more stable form that fuses with the body's cells. "The spike protein is not pathogenic. It is not a toxin," McLellan said. "I have not seen any data to support what Bridle claims."

FactCheck.org and Reuters both noted that Bridle's claims have spread online through fringe media, including anti-vaccine activist Robert F. Kennedy Jr. and white supremacist Hal Turner. Now, thanks to Ingraham, his anti-vaccine misinformation has been given a platform on Fox News' prime time.

Related

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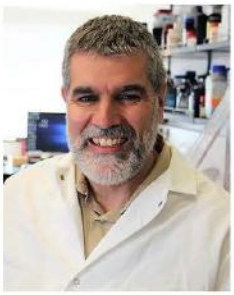
KATHERINE R. COSTIN

Dr Byram Bridle



Dr. Byram Bridle is an Associate Professor of Viral Immunology in the Department of Pathobiology at the University of Guelph.

His research program focuses on vaccine development for the prevention of infectious diseases and to treat cancers with the goal of translating findings into humans. He also studies fundamental aspects of immune response to viruses. Dr. Bridle received funding from the Ontario Government and the Government of Canada for pre-clinical development of vaccines against coronaviruses. He holds no commercial interests in his COVID-19-focused research. Dr. Bridle holds numerous grants for research into cancers and viral immunology. Since the COVID-19 pandemic was declared, he has been actively involved in providing fact-based answers to questions posed by the public to help them make fully informed decisions about their health. This has included ~250 media engagements ranging from radio shows, published articles, and appearances on televised news programs (including, but not limited to, W5, The West Block, CTV National News, and Fox News), spanning the local to international scope. He was also an invited keynote speaker for two international conferences about COVID-19, and has served as an invited member of numerous COVID-19-focused discussion panels.

Dr. Bridle has an excellent track record of following the science underpinning COVID-19 vaccines and predicting where this will lead. Here are two examples: Example #1. When Health Canada authorized the use of AstraZeneca's vaccine, Dr. Bridle, along with two colleagues, wrote an open letter requesting that this vaccine not be used, in part on the grounds that it was being investigated for a link to potentially fatal blood clots in many European countries. Less than two months later, Canada suspended the AstraZeneca vaccination program, because it was deemed to be too unsafe because of causing blood clots that cost the unnecessary loss of lives of Canadians. Example #2. In May 2021 Dr. Bridle publicly raised concerns about a potential link between mRNA-based COVID-19 vaccines and heart inflammation in young people, especially males due to his knowledge of an alarming biodistribution pattern for these vaccines. This is now a well-recognized problem that has been officially listed as a potential side-effect of the mRNA COVID-19 vaccines. It was also the subject of a recent Public Health Ontario Enhanced Epidemiological Summary Report highlighting the increased risk of myocarditis and pericarditis to young males following COVID-19 mRNA vaccination. Further, use of Moderna's COVID-19 vaccine has been discontinued for this reason in young males in most of Canada. During the declared pandemic, Dr. Bridle has published thirty-three peer-reviewed papers in high-quality indexed scientific journals, including three that are solely focused on COVID-19. He has also served as an expert witness for scientific matters related to COVID-19 in the Ontario Supreme Court of Justice, in service of the Court of Queen's Bench of Alberta, and for the High Court of New Zealand. His current messaging can be followed through 'Substack's COVID Chronicles' (https://viralimmunologist.substack.com/?utm_source=discover_search).

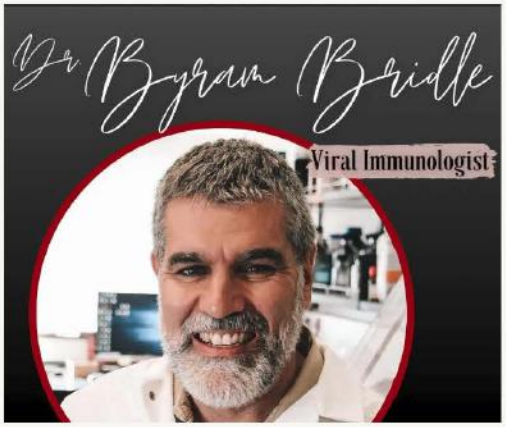
Sincerely,
Byram

CLICK TO GO BACK TO HOME

This is Exhibit R referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN



Dr. Byram Bridle joining Christine Anderson Tour in Toronto Feb 21, 2023

Leave a Comment / Guest Appearances, Toronto, Tour Updates / By wwcadtour

We are excited to share that Dr. Byram Bridle will be joining Christine on her tour at the Eglington Grand Theatre in Toronto on Feb 21, 2023.

I am an Associate Professor of Viral Immunology in the Department of Pathobiology at the University of Guelph. I specialize in vaccinology and am also leader of the Vaccine Task Force of the Canadian COVID Care Alliance's Scientific and Medical Advisory Committee.

My research program focuses on the development of vaccines to prevent infectious diseases and treat cancers, as well as studying the body's immune response to viruses. I teach several courses at the undergraduate and graduate levels on the topics of immunology, virology, and cancer biology. The overall aim of my research efforts is to develop safe and effective new immunotherapies for people. Indeed, one of my previous cancer vaccine strategies progressed into four human clinical trials.

I am also involved in training Canada's next generation of multidisciplinary researchers, especially in vaccinology. I received funding from the Ontario Government (COVID-19 Rapid Research Fund, Ministry of Colleges and Universities) and the Government of Canada (Pandemic Response Challenge Program, National Research Council of Canada) to conduct pre-clinical research with vaccines against COVID-19. I also hold numerous grants in support of my cancer research and basic viral immunology research programs, including but not limited to the Canadian Institutes for Health Research, Natural Sciences and Engineering Research Council of Canada, Terry Fox Research Institute, Canadian Cancer Society, and Cancer Research Society. I have received numerous teaching and research awards including the prestigious Terry Fox Research Institute's New Investigator Award and the Zoetis Award for Research Excellence.

I have served as an expert witness for court cases related to the science of COVID-19, including vaccines.

Since the COVID-19 pandemic was declared, I have been actively involved in providing fact-based, balanced, scientific answers to questions posed by the public to help them make fully informed decisions. This has included ~300 media engagements ranging from radio shows, published articles, and appearances on televised news programs, spanning the local to international scope. I have also been an invited keynote speaker at international conferences that focused on COVID-19 and have served as an invited member of numerous COVID-19-focused discussion panels.

Vaccinology is a highly specialized sub-discipline of immunology. I am called upon as an expert in this specialized field to comment on the critical importance of high-quality, well-validated, robustly safety-tested vaccines and I routinely promote their use. I support the concept of vaccine mandates in well-defined scenarios.

However, the definition of a vaccine had to be altered to allow the term to be applied to the current COVID-19 inoculations, which do not prevent infection, do not prevent disease (*i.e*, COVID-19), and do not stop transmission of SARS-CoV-2, which is the causative agent of COVID-19.

Get Your Tickets

Tour Updates

Viva Frei: From EU to WEF to Trudeau. Featuring Christine Anderson

The German Diplomat tour you won't hear about on left wing Media

European politician who supported Rolling Thunder to tour Canada (Western Standard)

Andrew Lawton Featuring Christine Anderson

Christine Anderson – Fighting For Freedom Documentary

nor can they transmit the targeted pathogen to others. After receiving these true vaccines, individuals not only do not need to be isolated, masked, or practice physical distancing, they are actively encouraged to interact with others.

Further, historically mandated vaccines were assessed for adverse events over long periods of time. The current COVID-19 inoculations had proper safety assessments ended after four months.

Notably, the six-month update report provided by Pfizer showed that adverse events were higher in the vaccinated group as compared to the placebo-treated controls, and demonstrated only modest absolute effectiveness against the original variant of SARS-CoV-2. The vaccine failed to reduce COVID-related hospitalizations and deaths and is now outdated and irrelevant in the context of the currently circulating variants of SARS-CoV-2.

Based on hundreds of peer-reviewed scientific publications, it is my professional opinion that the risk-benefit profile of SARS-CoV-2 inoculations currently being used around the world demands that mandates for these vaccines be rescinded to avoid exposing any more people, especially youth, to their enhanced risks.

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Dr. Crystal Luchkiw joining Christine Anderson Tour in Whitby, ON.

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Dr Eric Payne joining tour in Calgary

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Thanks for attending the tour event!

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Home Tour News Merch Contact

This is Exhibit S referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Ontario's 2023 sunshine list data is live. View below, or follow us on Twitter @SunshineList for new articles and interesting statistics including individual raises, and across employers, sectors and years.

Person | Employer | Sector | Year

1 optional columns selected | 10 records per page | Byram Bridle

Year	Employer	Name	Total
2023	University Of Guelph	Byram Bridle	$138,695.78
2022	University Of Guelph	Byram Bridle	$141,912.44
2022	University Of Guelph	Byram Bridle	$141,912.44
2021	University of Guelph	Byram Bridle	$136,881.92
2020	University of Guelph	Byram Bridle	$132,346.86
2019	University of Guelph	Byram Bridle	$125,964.00
2018	University of Guelph	Byram Bridle	$119,820.40
2017	University of Guelph	Byram Wayne Bridle	$115,851.51
2016	University of Guelph	Byram Bridle	$110,886.82
2015	University of Guelph	BYRAM BRIDLE	$111,859.24
All	Search Employer	Search Name	

Showing 1 to 10 of 11 entries (filtered from 1,445,448 total entries)

Previous 1 2 Next

This is Exhibit T referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

NSERC's Awards Database

Award Details

Back to Search Results

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Calming the Cytokine Storm: Elucidating Mechanisms Contributing to Toxic Inflammatory Responses to Viruses

Research Details

Application Id:	RGPIN-2021-04069		
Competition Year:	2021	**Fiscal Year:**	2022-2023
Project Lead Name:	Bridle, Byram	**Institution:**	University of Guelph
Department:	Pathobiology - Pathobiology	**Province:**	Ontario
Award Amount:	$32,000	**Installment:**	5 - 1
Program:	Discovery Grants Program - Individual	**Selection Committee:**	Genes, Cells and Molecules
Research Subject:	Animal biology	**Area of Application:**	Advancement of knowledge
Co-Researchers:	No Co-Researcher	**Partners:**	No Partners

Award Summary

Viruses induce type I interferons (IFNs) after cells sense viral pathogen-associated molecular patterns. These IFNs are detected by the IFN a/ß receptor (IFNAR), with downstream signaling inducing IFN-stimulated genes that facilitate clearance of viruses through mechanisms that include secretion of pro-inflammatory cytokines. Although this would suggest that a lack of type I IFN signaling might reduce pro-inflammatory cytokines, our results demonstrated the opposite. We developed a murine model of viremia in which hematopoietic cells lacked IFNARs. After these mice received intravenous injections of a highly attenuated, non-pathogenic vesicular stomatitis virus, they succumbed to massive cytokine storms in 18 hours. Out of 26 cytokines that were assessed, 25 reached abnormally high levels. Unexpectedly, and a focus of this application, was that cytokine dysregulation was more exaggerated in females compared to males. This is notable because there is a sex bias towards females in the incidence of many inflammation-mediated diseases. Preliminary results suggested neutrophils may have a predominantly regulatory role in this model, since pro-inflammatory cytokines became more dysregulated when these cells were depleted. Neutrophils can be sub-divided into two major phenotypes after separation in a density gradient. Hypothetically, one of these subsets may have a suppressive role during cytokine responses. Quantitative or qualitative differences in a suppressive subset of male neutrophils may confer resistance to dysregulated cytokine responses to viruses. We also propose a model of cell signaling cascades as a blueprint for testing a second hypothesis: interferon-stimulated response elements in sex-dependent promoters contribute to differential regulation of type I IFN-modulated cytokine responses to viruses. Objectives: 1. Assess quantitative versus qualitative differences in the immunoregulatory potential of subsets of neutrophils from males and females. 2. Identify other leukocytes, beyond neutrophils, that contribute to the virus-induced cytokine storm. 3. Identify mechanisms underlying sex-biased dysregulation of cytokine responses, including intracellular changes in cytokine signaling pathways during viral infection with and without IFNAR-blockade, and the role of host factors that may explain sex differences. Training the next generation of Canadian biomedical scientists in the interdisciplinary field of viral immunology is integral to this research. A need for this expertise has been highlighted by the protracted struggle for scientists to develop solutions for the coronavirus disease (COVID)-19 pandemic. This research should provide a mechanistic understanding of how sex can influence the link between impaired IFNAR-mediated signaling, cytokine overproduction, and the leukocyte subsets that are involved. This has relevance for a spectrum of sex-biased cytokine disorders ranging from life-threatening cytokine storms to excessive inflammation.

Date Modified: 2022-11-23

This is Exhibit U referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

New Record: 80 Grants to Outsmart Cancer

This year, the Cancer Research Society (the Society) will award 80 research grants valued at $9.6 million dollars; a new record for the organization! These grants are awarded to some of the most promising cancer research projects in Canada.

The Society receives hundreds of applications annually from researchers and clinicians in Canada, all seeking to obtain a $120,000 two-year grant for a cancer research project. Additionally, the Society awards special grants for the next generation of scientists, with the aim of supporting researchers who are at the start of their careers. This year, 4 researchers will receive this special grant. The Society is the only Canadian institution that has been offering such a grant and it has been doing so since 2013.

"As a leader in the field of cancer research in Canada, the Cancer Research Society is determined, now more than ever, to contribute to preventing, detecting and treating all types of cancer as demonstrated by the success of our annual grant competition. Each donor and partner can be sure that the donations collected will have a major impact for patients diagnosed with cancer, as well as for their loved ones."
– **Manon Pepin**, President and Chief Executive Officer of the Cancer Research Society

Discover some of the researchers' profiles and their innovative proposals by clicking here.

The financial support offered by the Society is made possible thanks to the generosity of thousands of donors and partners that co-finance particular grants such as Ovarian Cancer Canada, the Quebec Breast Cancer Foundation, the CURE Foundation, Canadian Institutes of Health Research - Institute of Cancer Research (CIHR-ICR), Canadian Institutes of Health Research - Institute of Musculoskeletal Health and Arthritis (IMHA) and The Leukemia and Lymphoma Society of Canada, as well as one of the grants funded by the Bank of Montreal.

The research projects were chosen using a rigorous process; committees composed of over 95 researchers and clinicians generously gave of their time to select the most promising projects from among the 350 applications received.

"The Society thanks all the experts who conducted the exhaustive analysis of the projects and congratulates

remarkable progress to outsmart cancer.

– **Dajan O'Donnell**, Director, Scientific Affairs and Partnerships, Cancer Research Society

Recall that the Society continues to finance grants and bursaries that began in previous years, in addition to particular special partnership research projects.

Here is the list of all the researchers selected.

Causes and prevention - the most effective approach to reducing the risk of developing the disease

Laura Anderson

McMaster University – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Identifying changes in cancer risk factors during the COVID-19 pandemic and predicting population risk

Parveen Bhatti

BC Cancer

Metabolomic markers to target prevention of postmenopausal breast cancer among overweight and obese women

Roberto Botelho

Ryerson University

The importance of phosphoinositide acyl regulation in cancer cell biology

Colin Collins

University of British Columbia

Identification and functional validation of genes driving therapy-induced dormancy in prostate cancer

Jean-François Côté

Montreal Clinical Research Institute – funded in partnership with The Quebec Breast Cancer Foundation

A deadly kinase promotes triple-negative breast cancer progression: defining the functions of PEAK1 protein complexes in tumour growth and metastasis

Michael Cox

University of British Columbia

How prostate cancer subtypes impact bone microarchitecture in metastatic lesions

Juliet Daniel

McMaster University

Kaiso, intestinal inflammation and colon cancer

John Di Guglielmo

University of Western Ontario

Targeting the type II TGFß receptor as a novel strategy for lung cancer

Savraj Grewal

University of Calgary

Oncogenic Ras signalling, and the metabolic regulation of hematopoietic tumours and whole-body physiology

David Hipfner

Pamela Hoodless

Nina Jones

Institute

A Drosophila model of cell-cell growth signalling through filopodia

partnership with the Environment-Cancer Fund™ of the Cancer Research Society and Read for the Cure

Defining epigenetic alterations in liver cancer at single cell resolution

partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Investigating the role of ShcD signalling in metastatic breast cancer

Mathieu Laplante

Institut universitaire de cardiologie et de pneumologie de Québec-Université Laval

Defining the role of ZNF768 overexpression in lung cancer development

Mohan Malleshaiah

Montreal Clinical Research Institute – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Single-cell analysis of the epithelial-to-mesenchymal transition in pancreatic cancer

Nathalie Rivard

Université de Sherbrooke

Exploring the role of Shp-2 in APC-mutated colorectal cancer

Rola Saleeb

St. Michael's Hospital – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Tracing the origin of kidney cancer from renal stem cells, understanding tumour pathogenesis for effective cancer prevention

Vivian Saridakis

York University

Identifying substrates of a critical E3 ligase in prostate cancer

Thomas Simmen

University of Alberta – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Rab32 promotes autophagic elimination of a metabolism control centre in breast cancer

Ulrich Tepass

University of Toronto

Mitosis as a driver of epithelial tumour progression

Hugo Wurtele

Centre de recherche de l'Hôpital Maisonneuve-Rosemont

Nascent chromatin structure as a modulator of cellular senescence in cancer

Detection – early detection is critical to improving patient outcomes

Scott Bratman

Pejman Jabehdar Maralani

Thanh Nguyen

Centre)

Methylated DNA as a novel early detection test for oral squamous cell carcinoma

Sunnybrook Research Institute

Advanced MRI for non-invasive mapping glioma stem cells in glioblastoma

Magnetic resonance spectroscopy as a non-invasive tool to diagnose and monitor gliomas

Neil Renwick

Queen's University – funded in partnership with the Steven E and Scott Drabin Research Fund

Evaluating microRNAs as tissue and circulating biomarkers for gastroenteropancreatic neuroendocrine neoplasms

John Trant

University of Windsor

Early identification of neuroendocrine-like prostate cancer using near-infrared light

Maruti Uppalapati

University of Saskatchewan – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Development of molecular imaging probes targeting Nectin4 (PVRL4) expression in cancer

Treatment – the development of new treatments, including targeted or personalized therapies

Tommy Alain

CHEO Research Institute – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Oral administration of Reoviruses for the treatment, prevention and early diagnosis of colorectal cancer

Vincent Archambault

Université de Montréal

Enhancing immunogenic nuclear assembly defects after cell division

Steve Bilodeau

Université Laval

Targeting the HSF1-regulated pathway in endocrine-resistant breast cancer to restore antiestrogen response

Peter Black

University of British Columbia

Assessment of IL-10 driven N-glycan branching as a regulator of CD8 T cell function in muscle-invasive bladder cancer

Jeanette Boudreau

Dalhousie University – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Immune evasion from natural killer cells by non-small cell lung cancer

Marie-Claude Bourgeois-Daigneault

Centre de recherche du CHUM – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Investigating the role of the immunoproteasome in the efficacy of oncolytic virotherapy

Byram Bridle

University of Guelph

Heat and cold adaptation of oncolytic rhabdoviruses to improve their clinical utility

John Burke

University of Victoria

Defining the molecular mechanism of activation of oncogenes in the PIP3/PDK1/Akt signalling pathway

Steven Chan

University Health Network (Princess Margaret Cancer Centre) – funded in partnership with The Leukemia & Lymphoma Society of Canada

Targeting SYK signalling to overcome resistance to IDH inhibitors in acute myeloid leukemia

Audrey Claing

Université de Montréal – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Role of ARF1 as a key GTPAse regulating immune surveillance in breast cancer

Marc Coppolino

University of Guelph – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Preventing breast cancer metastasis by disrupting MT1-MMP trafficking

Shoukat Dedhar

BC Cancer – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

The role of microbiota and immune microenvironment in breast cancer metastasis

Javier Di Noia

Montreal Clinical Research Institute – funded in partnership with The Leukemia & Lymphoma Society of Canada

An orphan deaminase as a novel source of chemotherapy resistance in cancer

Jean-Simom Diallo

Ottawa Hospital Research Institute

Discovery of novel liposarcoma antigens for immunotherapy development

Phedias Diamandis

University Health Network (Princess Margaret Cancer Centre) – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Exploring the role and therapeutic potential of hypoxia-driven AKAP12 expression in glioblastoma

Pierre-Olivier Fiset

Research Institute of the McGill University Health Centre – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Unlocking new options for patients with squamous cell carcinoma of the lung

Suresh Gadde

University of Ottawa – funded in partnership with The CURE Foundation

RNAi-chemotherapy combination nanomedicines for the effective treatment of triple-negative breast cancer

Etienne Gagnon

Institute for Research in Immunology and Cancer of the Université de Montréal – funded in partnership with The Leukemia & Lymphoma Society of Canada

Optimizing and benchmarking novel modular CARs targeting leukemia.

University of Alberta

Improving cervical cancer therapy with radiosensitizers for brachytherapy

Université de Montréal

Role of PAK2 in tumour angiogenesis and in the tumour microenvironment

Université de Montréal

The PGC-1 metabolic coactivators control immunosuppression and response to immunotherapy in melanoma

Martin Guimond

Centre de recherche de l'Hôpital Maisonneuve-Rosemont – funded in partnership with The Leukemia & Lymphoma Society of Canada

The effect of EGFL7 on graft-versus-host disease and graft-versus-leukemia effect

Patrick Gunning

University of Toronto – funded in partnership with The Leukemia & Lymphoma Society of Canada

Development of selective HDAC inhibitors for the treatment of hematological malignancies

Xi Huang

SickKids

Tissue stiffness heterogeneity is governing the replicative potential and chemosensitivity of brain tumour-initiating cells

Carolina Ilkow

Ottawa Hospital Research Institute – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Understanding the role of adipose tissue and fat cells in cancer virotherapy resistance

Valentin Jaumouillé

Simon Fraser University – funded in partnership with The Leukemia & Lymphoma Society of Canada

Understanding and exploiting the mechanobiology of macrophages to promote clearance of lymphoma cells by phagocytosis

Éric Lévesque

CHU de Québec - Université Laval

Aberrant UGT2B28 androgen inactivation pathway predicts prostate cancer progression

William Lockwood

BC Cancer

Managing EGFR inhibitor resistance in lung adenocarcinoma through drug holiday- induced hyperactivation of oncogenic pathways

Rachid Mazroui

Université Laval

Role of DDX3-ALKBH5 interaction in ATF4 mRNA translation: implications in chemoresistance

Judith Andrea McCart

Mount Sinai Hospital

Investigating the role of innate immune responses in the anti-tumor effects of oncolytic vaccinia virus

Jane McGlade

SickKids – funded in partnership with The Leukemia & Lymphoma Society of Canada

GADS dependent signalling

Peter Metrakos

Research Institute of the McGill University Health Centre

Role of innate immune cells in the development of vessel co-

Eric Milot

Centre de recherche de l'Hôpital Maisonneuve-Rosemont – funded in partnership with The Leukemia & Lymphoma Society of Canada

response in acute lymphocytic leukemia

Christopher Mueller

Queen's University – funded in partnership with the Helen Lenore Bailey Fund

A Liquid Biopsy for Monitoring Treatment Response to Immunotherapy in NSCLC

Yvonne Myal

University of Manitoba – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

The role of prolactin-inducible protein (PIP) in promoting breast cancer metastasis in the lung

Michael Olson

Ryerson University – funded in partnership with Ovarian Cancer Canada

MRCK in high-grade serous ovarian cancer: defining pathways promoting cell proliferation and mechanisms of drug resistance

Ayman Oweida

Université de Sherbrooke – funded in partnership with the Canadian Institutes of Health Research - Institute of Musculoskeletal Health and Arthritis

Targeting immunosuppressive chemokines for enhanced response to radiotherapy in squamous cell cancers

Morag Park

McGill University – funded in partnership with The Quebec Breast Cancer Foundation

B7-H4 as a therapeutic target in poor-outcome triple-negative breast cancers

Moutih Rafei

Université de Montréal – funded in partnership with The Leukemia & Lymphoma Society of Canada

Enhancing endosome-to-cytosol import of antigen to augment antitumoral immunity

Mir Munir Rahim

University of Windsor – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Immunosurveillance of breast cancer by innate immune cells

Gregor Reid

University of British Columbia

Prospective characterization of relapse-driving blasts in acute lymphoblastic leukemia

Francis Rodier

Centre de recherche du CHUM – funded in partnership with The Leukemia & Lymphoma Society of Canada

Evaluating new pharmacologically targetable molecular hallmarks of premature aging in childhood blood cancer survivors

Philippe Roux

Institute for Research in Immunology and Cancer of the Université de Montréal

Targeting CDK12 to overcome melanoma chemoresistance

Tanveer Sharif

University of Manitoba – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Therapeutic exploitation of

Trevor Shepherd

University of Western Ontario

Therapeutic targeting of ULK1, a crucial regulator of autophagic stress in metastatic ovarian cancer

Maya Shmulevitz

University of Alberta – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Overcoming inactivation of oncolytic reovirus by proteases in breast tumour microenvironments

Matthew Smith

Université de Montréal

Structure-function analysis of a novel KRAS effector complex and its role in metastasis

Sebastien Talbot

Université de Montréal – funded in partnership with the Canadian Institutes of Health Research - Institute of Cancer Research

Tumour-innervating PDL1-positive neurons block cancer immunosurveillance

Laura Trinkle-Mulcahy

University of Ottawa

Contribution of a novel nuclear stress response to pro-survival signalling and stress adaptation

Jim Uniacke

University of Guelph

Ribosomal protein S24 isoforms in adaptation to hypoxia and cancer progression

Barbara Vanderhyden

Ottawa Hospital Research Institute

Defining LATS1/2 as drivers of ovarian cancer and its immune suppressive microenvironment

Franco Vizeacoumar

University of Saskatchewan – funded in partnership with the Charlotte Légaré Memorial Fund

Developing novel targeted therapies for telomerase-overexpressing pancreatic cancers

Donna Wall

SickKids

Revisioning autologous transplant in pediatric solid tumors

Gelareh Zadeh

University Health Network (Princess Margaret Cancer Centre)

Uncovering the oncogenic potential of CIC-fusions and JAK/STAT1/3 activation in CIC-rearranged sarcomas

Recipients of the Scholarship for the Next Generation of Scientists (SNGS)

Barbara Grünwald

University Health Network (Princess Margaret Cancer Centre) – funded in partnership with BMO Financial Group

Part 1 : Fibroblast populations determining regional microenvironmental states in pancreatic cancer

Joshua Moreau

University of California, San Francisco

Part 1 : Dissecting the functional role of layilin on tumour-infiltrating T cells
Part 2 : Untangling the duelling pro- and anti-cancer potential of tumour-infiltrating B cells

Dominic Roy

McGill University –Rosalind and Morris Goodman Cancer Institute

Part 1 : Metabolic regulation of oncolytic virotherapy
Part 2 : Targeting glycolysis to improve oncolytic virotherapy

pancreatic cancer stroma

Dheva Setiaputra

Lunenfeld-Tanenbaum Research Institute

Part 1 : Uncovering the molecular mechanisms of DNA double-strand break repair
Part 2 : Dissecting DNA double-strand break repair and its role in cancer biology

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This is Exhibit V referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

vaccines

MDPI

Review

Maternal COVID-19 Vaccination and Its Potential Impact on Fetal and Neonatal Development

Niel A. Karrow [1,*], Umesh K. Shandilya [1], Steven Pelech [2], Lauraine Wagter-Lesperance [3], Deanna McLeod [4], Byram Bridle [3] and Bonnie A. Mallard [3]

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[3] Department of Pathobiology, University of Guelph, Guelph, ON N1G 2W1, Canada; lwagterl@uoguelph.ca (L.W.-L.); bbridle@uoguelph.ca (B.B.); bmallard@ovc.uoguelph.ca (B.A.M.)
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Abstract: Vaccines have been developed under accelerated timelines to combat the COVID-19 pandemic caused by the SARS-CoV-2 coronavirus. Although they are considered the best approach for preventing mortality, when assessing the safety of these vaccines, pregnant women have not been included in clinical trials. Thus, vaccine safety for this demographic, as well as for the developing fetus and neonate, remains to be determined. A global effort has been underway to encourage pregnant women to get vaccinated despite the uncertain risk posed to them and their offspring. Given this, post-hoc data collection, potentially for years, will be required to determine the outcomes of COVID-19 and vaccination on the next generation. Most COVID-19 vaccine reactions include injection site erythema, pain, swelling, fatigue, headache, fever and lymphadenopathy, which may be sufficient to affect fetal/neonatal development. In this review, we have explored components of the first-generation viral vector and mRNA COVID-19 vaccines that are believed to contribute to adverse reactions and which may negatively impact fetal and neonatal development. We have followed this with a discussion of the potential for using an ovine model to explore the long-term outcomes of COVID-19 vaccination during the prenatal and neonatal periods.

Keywords: COVID-19; SARS-CoV-2; vaccines; fetal development; neonatal development

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1. Introduction

Vaccines are a key strategy for preventing and controlling endemic and emerging diseases of both humans and livestock. In the case of COVID-19, which is caused by the zoonotic SARS-CoV-2 coronavirus, vaccines have been designed and produced under accelerated timelines, in part due to programs such as Operation Warp Speed [1]. Never before have vaccines been developed and made it to Phase III clinical trials in such a short period of time. The viral vector "Sputnik V" vaccine was the first to be registered in August 2020. The Oxford/AstraZeneca viral vector vaccine was later approved for use in the UK vaccination program in December 2020, and in that same month, the Pfizer–BioNTech mRNA vaccine was issued "emergency use authorization" by the US Food and Drug Administration, and was approved for individuals of 16 years of age and older in May 2021.

As of 23 October 2021, four different COVID-19 vaccines have been approved for use in Canada: Oxford/AstraZeneca's Vaxzevria (Cambridge, UK), Pfizer–BioNTech's Comirnaty (New York, NY, USA), Moderna's Spikevax (MA, USA), and Johnson & Johnson's Janssen (New Brunswick, NJ, USA) [1]. At this time, approximately 77% of the Canadian population that was 12 years or older had received at least one dose, and 84% was fully vaccinated, with most receiving the Pfizer–BioNTech and Moderna vaccines. Approximately 10% had

received combinations of different vaccines, despite warnings against this "dangerous trend" by the World Health Organization (WHO), due to a lack of immunogenicity and safety data [2].

In terms of females, approximately 79% of Canadian females had received at least one dose and 75% were fully vaccinated at this point in time. Females mount a stronger immune response to vaccination than males, which can also make them more susceptible to adverse vaccine reactions (AVR) [3]. The number of COVID-19 vaccinated pregnant women in Canada is currently unknown, but it can be approximated using the numbers of births in Canada from 2001–2020 [4]. If we estimate 370,000 births per year, one can conservatively approximate that 243,581 pregnant Canadian women received at least one COVID-19 vaccine dose and that 231,248 were fully vaccinated within the 10-month vaccine rollout period based on the percentages of vaccinated females above. There is a concerted global effort underway to encourage pregnant women to get vaccinated despite the lack of safety data for this demographic [5], the rationale being that pregnant women who get COVID-19 are more likely to get critically ill and have adverse fetal/neonatal outcomes [6]. However, this rationale is not supported by all studies [7], and vaccination without long-term safety data from a randomized clinical trial and close medical oversight does not follow a precautionary principle, which is the standard of care in this group.

In the USA, 133,000 participants of the V-safe COVID-19 Vaccine Pregnancy Registry have indicated that they were pregnant at the time of vaccination, and the US Centers for Disease Control and Prevention (CDC) is currently enrolling eligible participants and analyzing data (i.e., pregnancy outcomes such as miscarriage and stillbirth, pregnancy complications such as preeclampsia and gestational diabetes, and problems with newborns such as preterm delivery, poor growth or birth defects) to better understand how COVID-19 vaccination may affect pregnant women [8]. A widely cited preliminary study of the V-safe and Vaccine Adverse Event Reporting System (VAERS) data [9] suggested that COVID-19 mRNA vaccines were safe for pregnant women [10]; however, an error was found in their analysis that undermined the original conclusion of safety in the context of spontaneous abortions [11], forcing a correction by these authors [12]. While these post-hoc data analyses of extreme clinical outcomes are important for assessing vaccine safety during pregnancy, they do not include more subtle multi-organ developmental changes that would be expected to occur in the fetus during an AVR, and these could lead to an increased risk of disease according to the Developmental Origins of Health and Disease (DOHaD) Hypothesis [13].

We have actually been advised to "feel positive about feeling bad" after receiving a COVID-19 vaccine [14]. However, the desired goal of these vaccines, to drive an anti-viral cell-mediated immune response against SARS-CoV-2 (i.e., the pro-inflammatory cytokines tumor necrosis factor (TNF) α, interleukin (IL)-1, IL-6, and Type I and II interferons (IFNs)), can also lead to adverse fetal outcomes [15,16].

2. Lipid Nanoparticles (LNPs) in the COVID-19 mRNA Vaccines

COVID-19 vaccine development has truly been unprecedented. Not only have vaccines been rapidly produced and approved for use, but this is the first time a coronavirus vaccine has ever been attempted for use on humans. Moreover, vaccines against infectious pathogens have not previously been created using the novel technologies that were used to develop the current emergency use COVID-19 vaccines. The mRNA vaccine platforms (Pfizer–BioNTech and Moderna) contain a genetically modified mRNA sequence encoding the immunogenic SARS-CoV-2 spike protein, which is used by the virus to invade host cells. They also contain a novel lipid nanoparticle (LNP) carrier system that allows for efficient endocytosis of the mRNA cargo by host cells. These LNPs possess adjuvant-like properties, both inflammatory and mRNA stabilizing, which is why conventional adjuvants are not required for these vaccines [17]. Nevertheless, the RNAs are not very stable when stored below $-80\ ^{\circ}C$, and rapidly degrade at body temperature. The LNPs are comprised of ionizable cationic lipids, phospholipids, cholesterol and polyethylene glycols (PEGs),

which are used to control the LNP size (60–100 nm), prolong circulation time and prevent LNP aggregation during vaccine storage [18]. Concerns were raised years ago regarding the safety of LNPs due to their biodistribution. For example, they were found to disperse to the ovaries in experimental mice [19]. Pfizer's own pharmacokinetic studies of a surrogate vaccine containing ALC0315 and ALC0159 LNPs demonstrated that they dispersed over a 48 hours period to many rat endocrine and immune organs including the ovaries, adrenals, bone marrow, liver and spleen [20].

Very little is known about how LNP particle components are metabolized by the human body. Thus, further research must be completed, or information on studies from the companies that manufacture LNP components must be made available on Safety Data Sheets to indicate how these LNPs degrade into smaller catabolites. Research must also be conducted into how LNP components and their catabolites are distributed, retained and excreted. A critical component of the LNPs in both mRNA vaccines is the pegylated lipid, composed of a PEG unit with an average molecular mass of 2000 Da—DMG2000 in the Moderna and ALC-0159 in the Pfizer–BioNTech vaccine. Interestingly, smaller PEG molecules have been studied as a possible means for both inducing retinopathy and as a means for drug delivery to the eye. C57BL/6 mice were administered an intraocular injection of PEG8 to induce choroid neovascularization (CNV) after complement activation [21], and may serve as a model for studying macular degeneration of the retina. Dutch belted rabbits injected with PEG400 were reported to have retinal degeneration and atrophy 5 days post injection [22]. Both of these studies demonstrate that small sized PEGs can be toxic and, though helpful as a model for disease, indicate that pegylated lipids are an unsuitable method for intraocular drug delivery. As a follow up to this, two recent case reports published in the USA reveal a possible association with mRNA vaccines and damage to the retina. Subramony et al. [23], for example, reported a case of bi-lateral retinal detachment in a healthy 22-year-old after vaccination with the Moderna mRNA vaccine. This individual had no health issues, but upon ophthalmologic exam, was determined to have lattice degeneration. Post-vitreous retinal detachment is common in >50-year-olds due to the liquefied vitreous pulling away from the retina, but not in younger patients. Lattice degeneration by itself in this individual is unlikely to cause a retinal detachment; thus some other mechanism must have caused the retina to detach around this area of lattice. Fowler et al. [24] reported acute onset central serous retinopathy in a 33-year-old healthy male post-Pfizer—BioNTech vaccination. Given the known factors that cause acute central serous retinopathy, the author speculated on a few possible mechanisms as to why this occurred, including increased serum cortisol and free extracellular RNA—which can cause increased permeability of choroid endothelial cells—but also suggested that PEG may be involved, and mentioned the fact that PEG8 has been shown in mice to induce central serous retinopathy via the complement pathway. Without evidence of how catabolites of PEG in COVID-19 vaccines circulate and are excreted, one could hypothesize that PEG2000 molecules are broken down into smaller sizes, which could permit them to enter into the vasculature of immune-privileged tissues such as the eye and cause pathology.

LNPs are bioactive and the possibility of immunotoxicity has been raised. The innate immune system, for example, is activated when phagocytic cells (i.e., dendritic cells, macrophages, Kupffer cells, monocytes, mast cells and granulocytes) come into contact with LNPs, which are recognized as danger signals by host cell toll-like receptors (TLRs). Ligation of LNPs to these TLRs triggers the induction and release of abnormally high levels of pro-inflammatory and anti-inflammatory cytokines, referred to as cytokine release syndrome. LNPs can also activate serum complement, resulting in complement activation-related pseudoallergy (CARPA), which can lead to anaphylactic shock [25]. A recent pre-print study demonstrated that, when LNPs were injected intradermally into mice, inflammatory, pro-apoptotic, necroptotic and IFN gene pathways were induced, and when these LNPs were administered intranasally, 80% of mice died within 24 h [26].

PEGs have been previously used in both cancer immunotherapies and to deliver cytotoxins throughout the body. They have also been used to dampen cytokine and

complement activation triggered by LNPs, but an optimal concentration of PEG is required to both maximize LNP protection from the immune system and to ensure thatthe LNP cargo remains bioactive [25]. PEGs were thought to have inert characteristics; however, it is now widely appreciated that they possess potent immunogenic properties. Exposure to PEG can result in the production of anti-PEG immunoglobulin (Ig)M and IgG, which can activate the complement system and result in anaphylaxis [27]. Anaphylaxis is one AVR that is associated with the COVID-19 mRNA vaccines [28], and for the Pfizer–BioNTech vaccine, the risk is 1:100,000 [29]. Since PEG is commonly used in consumer products, a considerable number of people may have already been sensitized to PEG and may therefore have pre-existing anti-PEG antibodies prior to COVID-19 vaccination [25]. Following endocytosis of the LNPs, PEGs can also freely interact with IgE antibodies that are bound to Fc receptors on mast cells and granulocytes; this can lead to Fc cross-linking that immediately triggers cellular degranulation, also resulting in anaphylaxis [30]. While anaphylaxis during pregnancy is typically a rare event, a recent study has reported severe outcomes for infants from mothers with anaphylaxis [31], which should alert us to potential fetal/neonatal outcomes resulting from vaccine-induced maternal anaphylaxis.

3. Viral Vector COVID-19 Vaccines

The COVID-19 viral vector vaccines (Oxford/AstraZeneca, Janssen, and Sputnik V) rely on adenovirus DNA vectors as carriers for the genetic information coding for the SARS-CoV-2 spike protein. Following intramuscular injection, the adenovirus invades host cells via receptor-mediated endocytosis. Its DNA is carried to the nucleus, and the host cell machinery then transcribes and translates it into spike proteins. Since, typically, 30 or more mRNA copies can be transcribed from a single DNA copy of this gene, this allows for a more marked amplification of total spike proteins than can be produced in the RNA-based vaccines.

The most commonly described side effects following the Oxford/AstraZeneca vaccination are injection site erythema, pain, swelling, fatigue, headache, fever and lymphadenopathy. However, in March 2021, vaccine-induced prothrombotic immune thrombocytopenia (VIPIT), also referred to as thrombosis–thrombocytopenia syndrome (TTS) or vaccine-induced immune thrombotic thrombocytopenia (VITT), was first reported for the Oxford/AstraZeneca vaccine. This should not be surprising, since thrombocytopenia has been consistently reported as an outcome of administering adenovirus vectors [32]. In April 2021, similar reports started to appear for the Janssen vaccine. Females less than 60 years of age are at the greatest risk of VIPIT within 5–30 days post-vaccination, and the estimated risk is 1:25,000 and 1:500,000 for the Oxford/AstraZeneca and Janssen vaccines, respectively [33]. In light of this AVR, many countries temporarily halted the use of these vaccines, but they were later reinstated, because the risk of COVID-19 was deemed greater than the risk of VIPIT. A number of hypotheses have been proposed to explain the potential mechanisms of VIPIT, including antibodies acting against platelet factor 4 (PF4), interactions between the adenovirus and platelets, cross-reactivity of SARS-CoV-2 spike proteins with PF4 (i.e., molecular mimicry), interactions between spike proteins and platelets, and platelet expression of adenoviral proteins [34]. With regards to pregnancy, a case study of immune thrombocytopenia was reported in a woman with mild COVID-19, and her newborn daughter who was COVID-19 free also experienced a decrease in platelet count that was resolved within 3 weeks postpartum [35]. In another study, a young COVID-19 positive woman who delivered a stillbirth at 29 weeks into gestation was also diagnosed with thrombotic thrombocytopenic purpura [36]. Interestingly, new-onset immune thrombocytopenia post-mild COVID-19 has been reported during the pandemic [37], and also following Pfizer–BioNTech vaccination [38], which indicates that the etiology of this condition is more complex than can be explained by the adenoviral vectors alone.

Rare neurological manifestations, such as Guillain–Barre syndrome (GBS), have also been reported to be associated with the COVID-19 adenovirus vector vaccines [39–43]. GBS is an acute inflammatory, demyelinating polyneuropathy characterized by progressive

muscle weakness that is often self-resolving. However, in severe cases where respiratory muscles are compromised, it can be life-threatening and patients will require assisted mechanical ventilation [44], which has been reported to increase the risk of premature birth [45]. GBS is most commonly triggered by molecular mimicry following a gastrointestinal or respiratory illness. Mohkhedkar et al. [46] recently provided evidence to support the involvement of molecular mimicry in COVID-19 by identifying autoantibodies in cerebral spinal fluid from a GBS-diagnosed patient with COVID-19. It has also been proposed that pro-inflammatory cytokines and hypoxia may also contribute to COVID-19 related neuronal damage [43].

In addition to concerns about the adenovirus vectors, the Oxford/AstraZeneca vaccine also contains polysorbate 80 (Tween 80), which helps to stabilize the vaccine. This synthetic non-ionic surfactant has been previously used in various drug formulations [47]. However, polysorbate 80 is cross-reactive with PEG, so anti-PEG antibodies may also trigger an IgE-mediated hypersensitivity reaction to polysorbate 80 [28,48].

Lastly, concerns have been raised about the potential fate of foreign DNA in human cells, sourced from either an adenovirus vector, or reverse-transcribed mRNA coding the spike protein. While this is theoretically possible based on gene therapies, the likelihood of SARS-CoV-2 mRNA being reverse-transcribed into DNA and then integrating within the host genome is equally plausible during COVID-19 [49]; thus, the benefits of the COVID-19 vaccines for now are thought to outweigh the potential risk of DNA integration [50].

4. Bioactivity of the SARS-CoV-2 Spike Protein

The main host target receptor for the SARS-CoV-2 spike protein is angiotensin-converting enzyme 2 (ACE2), which is involved in maintaining blood pressure and vascular remodeling, and is expressed on adipocytes [51], other cells at mucosal surfaces, and in the vasculature, heart, kidneys, pancreas and brain [52]. ACE2 is also expressed within placental tissues [53], and is involved in regulating fetal myocardial growth and lung and brain development [54]. A recent pre-print study showed that blocking ACE2 with an anti-ACE2 antibody reduced placental SARS-CoV-2 infection [55]. This is one of a number of studies that have demonstrated that the placenta is susceptible to SARS-CoV-2 [54,56], and may also be responsive to spike proteins, which have been identified at low concentrations in plasma from recipients of the Moderna vaccine [57].

A large number of studies have provided evidence that the SARS-CoV-2 spike protein is bioactive, and that ligation of the spike protein to ACE2 explains some of its bioactivity. A study by Lei et al. [58], for example, demonstrated that the spike protein down-regulated ACE2 in Syrian hamster vascular endothelial cells, which led to inhibited mitochondrial function and cell damage. In a later in vitro study, however, the spike protein was shown to upregulate bronchial epithelial cell ACE2 expression via activation of the Type I IFN signaling pathway [59]. These two studies indicate that the effect of this spike protein on ACE2 expression is tissue or species-specific. A recent study using mice and human umbilical cord blood demonstrated that ligation of recombinant spike protein to ACE2 can activate Nlrp3 inflammasome assembly, resulting in uncontrolled inflammation leading to pyroptotic cell death [60]. Ropa et al. [61] demonstrated that hematopoietic stem cells from human umbilical cord blood express ACE2 and were adversely affected by spike protein in terms of their ability to proliferate and expand into progenitor cells. Ropa et al. proposed that this could explain the reduced numbers of circulating lymphocytes and platelets that are observed in COVID-19 patients [61]. Using wild type and transgenic mice expressing human ACE2, Biancatelli et al. [62] recently demonstrated that intratracheal administration of the spike protein S1 subunit induced alveolar inflammation and acute lung injury and altered lung vascular permeability, leading to an ACE2-dependent systemic cytokine storm. Suzuki et al. recently demonstrated that the spike protein S1 subunit (Val-16-Gln-690), but not the ACE2 receptor binding domain (Arg-319-Phe-541), elicited mitogen-activated protein kinase (MEK/ERK) signaling in human pulmonary artery smooth muscle and endothelial cells [63]. These authors proposed that this growth factor/hormone-like cell

signaling contributes to the hyperplasia and/or hypertrophy of vascular smooth muscle and endothelial cells in patients with COVID-19 and may also possibly explain some of the AVRs associated with COVID-19 vaccines [63]. By combining their knowledge of the SARS-CoV-2 spike protein, and the work of Chen et al. [64] on the SARS-CoV-1 spike protein conducted on human pneumocytes, Suzuki proposed that the spike protein functionally converts ACE2 from a peptidase to a functional cell membrane signaling receptor (Figure 1).

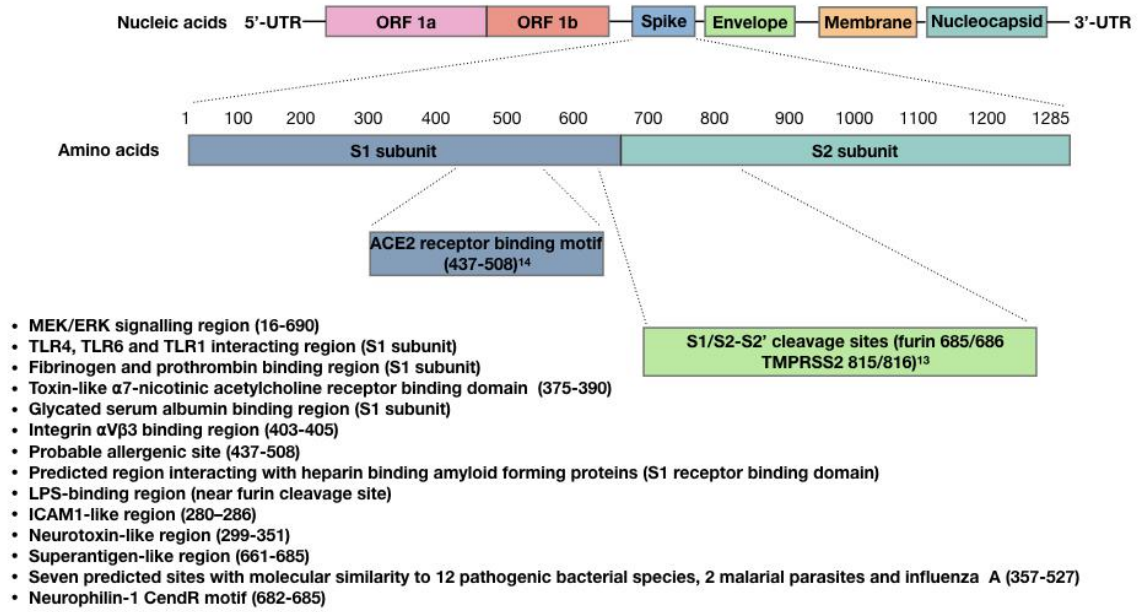

Figure 1. Sites within the SARS-CoV-2 spike protein S1 subunit and receptor binding domain, showing confirmed or predicted bioactivity.

A number of studies have shown that the SARS-CoV-2 spike protein also possesses ACE2-independent bioactivity. Nader et al. [65] for example, found that in addition to ACE2, SARS-CoV-2 can also attach to, invade and damage host cells via αVβ3 integrin adhesion molecules, which are highly expressed on vascular endothelial cells. These authors demonstrated that an arginine–glycine–aspartic acid mutation (RGD motif) in this spike protein has uniquely allowed SARS-CoV-2 to acquire this function. Since the RGD motif is located adjacent to the ACE2 receptor-binding motif (Figure 1), this could allow SARS-CoV-2 to bind to cells lacking ACE2 and to potentially enhance binding to cells expressing both ACE2 and αVβ3 integrin. Interestingly, αVβ3 integrins are also expressed on platelets and contribute to platelet activation and aggregation [66]. Shen et al. showed that SARS-CoV-2 interacts with platelets to influence their function and promote dysregulated coagulation [67]; they proposed an ACE2-independent mechanism for this, because the expression of ACE2 is uncertain in platelets and their progenitor megakaryocytes [68]. It is possible that αVβ3 integrin is involved in SARS-CoV-2 interactions with platelets.

In silico analysis of the S1 subunit of the SARS-CoV-2 spike protein has revealed molecular docking sites for TLRs, including TLR1, TLR4 and TLR6; interactions with spike protein were the strongest for TLR4 [69] (Figure 1). An in vitro study performed by Shirato and Kizaki [70] demonstrated that the spike protein S1 subunit induced murine peritoneal macrophages to secrete pro-inflammatory cytokines via TLR4 signaling and that the response was attenuated using a TLR4 antagonist. TLR4 is also highly expressed in platelets, and when bacterial lipopolysaccharide (LPS) binds to TLR4, it can result in thrombocytopenia and the accumulation of platelets in the lungs [71]. Ouyang et al. [72] recently demonstrated that SARS-CoV-2 spike protein can also bind to bacterial LPS, and this spike protein-LPS interaction was shown to boost monocyte NF-κB activation and cytokine responses in vitro, as well as NF-κB activation in vivo [73]. Petruk et al. predicted

the LPS interacting region to be within the proximity of the spike protein S1/S2 furin cleavage site (Figure 1), and proposed that spike protein–LPS interactions may in part explain the increased risk of severe COVID-19 caused by comorbidities.

The SARS-CoV-2 spike protein can also interact with other proteins. Grobbelaar et al. [74] demonstrated that when the spike protein S1 subunit was added to platelet-poor plasma, it interacted with and structurally modified plasma proteins β and γ fibrinogen, complement 3 and prothrombin, which made them more resistant to trypsinization. These authors proposed that this may contribute to the hypercoagulation associated with COVID-19 and may impair clot breakdown during fibrinolysis (Figure 1). The SARS-CoV-2 spike protein can also bind with high affinity to glycated human serum albumin. This may allow SARS-CoV-2 to evade the detection of its receptor-binding domain (RBD) by neutralizing antibodies (Figure 1); however, it can also lead to albumin depletion and may contribute to fluid tissue–vascular imbalance that can give rise to septic shock [75]. Also found within the RBD of SARS-CoV-2 and SARS-CoV-1 spike proteins is a "toxin-like" epitope that shares homology to snake venom α-bungarotoxin [76], which is a highly specific blocker of nicotinic acetylcholine receptors. Lagoumintzis et al. [77] hypothesize that the SARS-CoV-2 spike protein may block the cholinergic anti-inflammatory pathway, allowing for uncontrolled inflammation to occur during COVID-19.

The SARS-CoV-2 spike protein can also bind to the b1b2 domain of the neuropilin-1 receptor (NRP-1) [77], which normally interacts with vascular endothelial growth factor-A (VEGF-A) in neurons. ACE2 is not present in most neurons [78], although reports of neurological symptoms are common in COVID-19 patients [79]. Interestingly, interactions between the polybasic 682RRAR685 amino acid sequence, termed the "C-end rule" (CendR) motif (Figure 1), with NRP-1 potentiates SARS-CoV-2 entry into host cells [80]. This CendR motif is not conserved in either SARS-CoV-1 or Middle East respiratory syndrome coronavirus (MERS-CoV), and it is hypothesized that a "silencing" of pain through subversion of VEGF-A/NRP-1 signaling may underlie increased disease transmission in asymptomatic individuals [81].

There are three other regions of interest within the spike protein RBD that may also contribute to spike protein bioactivity. The first region is predicted with high probability to be an allergenic sequence [30]. This region could therefore contribute to anaphylaxis in some patients that have received viral vector and/or mRNA COVID-19 vaccines. The second RBD region of interest potentially allows the spike protein to bind to amyloid-forming heparin-binding proteins, which could lead to accelerated aggregation of amyloid proteins within the brain [82]. This supports Classen's concern that COVID-19 vaccines could potentially induce prion disease [83]. The third region of interest within the RBD contains seven predicted molecular sites that share similarities to different toxins or virulence factors from 12 different bacterial species, 2 malarial parasites and influenza A [84] (Figure 1).

There is one final aspect of this spike protein that warrants consideration regarding its bioactivity, and this stems from the hypothesis that COVID-19-associated multi-system inflammatory syndrome in children (MIS-C) and the cytokine storm observed in adult patients with severe COVID-19 is mediated by spike protein superantigenic activity. Rivas et al. [85] have built on this hypothesis, first by drawing parallels between these two COVID-19 conditions and toxic shock syndrome (TSS). The superantigen *Staphylococcus* Enterotoxin B (SEB), associated with TSS, is a biotoxin that causes polyclonal T-cell activation and proliferation, which leads to massive production of pro-inflammatory cytokines. These researchers used structure-based computer modelling to discover an SEB-like sequence (glutamic acid661–arginine685) near the spike protein S1/S2 cleavage site that exhibits high binding affinity to both the T-cell receptor (TCR) β chain and co-stimulatory molecule CD28 [86] (Figure 1). They also identified several neurotoxin-like sequences within the spike protein; one (threonine299–tyrosine351) also displayed a high tendency to bind to the TCR, and another is an ICAM1-like region (aparagine280–threonine286) that is predicted to stabilize interactions between the spike protein and the TCR. These researchers also demonstrated TCRV/β skewing of the T cell response in COVID-19 patients with more

severe and hyper-inflammatory clinical courses, which is consistent with spike protein superantigen activity. Additionally, they showed that the SARS-CoV-2 mutation aspartic acid839–tyrosine predictably enhanced binding affinity of the spike protein to the TCR, and later this group also provided evidence that a repurposed anti-SEB antibody could prevent SARS-CoV-2 infection in vitro [87].

Collectively, the diverse bioactivity of the SARS-CoV-2 spike protein makes this an ideal target for the immune system to neutralize the virus, and all the current COVID-19 vaccine platforms have focused on this spike protein because it is highly immunogenic [88]. However, we should also be cognizant of these bioactive properties when designing COVID-19 vaccines to ensure that only nontoxic immunogenic portions of the spike protein are expressed, and that their expression is both temporally and spatially limited and does not provide selection pressure driving viral mutation. The mRNA vaccines have been designed to allow a host cell to express the spike protein in its cell membrane [89], and the expression of the spike protein throughout the body is dependent on the biodistribution of LNPs—which primarily relocate to the spleen and liver, but have also been found in various other tissues [17,20]. We currently have no idea how long spike proteins are expressed by different host cells and in what tissues spike protein expression can occur because biodistribution studies on the spike protein have not been carried out to date [20]. The mRNA sequence has also been modified by manufacturers, with the addition of proline residues at positions 986 and 987, which could allow them to reside longer in the plasma membrane [17]. A recent pre-print by Patterson et al. [90] indicated that a subset of monocytes from COVID-19 patients contained SARS-CoV-2 S1 mRNA and proteins for as long as 15 months post-acute infection. This raises the possibility of the spike protein being expressed by maternal immune cells in colostrum and milk from COVID-19 positive mothers; thus, the biodistribution of spike mRNA and protein could be especially relevant during lactation. A recent study by Golan et al. [91] suggested that biodistribution of mRNA to milk during lactation is not a concern, as none was detected in milk from 6 mothers 4–48 h post-Pfizer–BioNTech and Moderna vaccination. However, a study demonstrated that following COVID-19 mRNA vaccination, exosomes expressing spike protein could be detected in plasma up to 4 months post-vaccination [92], which is concerning because we, and others [93,94], have shown that exosomes can be shed in bodily fluids such as colostrum and milk.

Lastly, Zhang et al. [49] have provided evidence that SARS-CoV-2 sequences can become integrated into human genomic DNA, and Seneff and Nigh speculated that retrotransposons in sperm and embryos could theoretically copy and paste SARS-CoV-2 cDNA into the fetal genome, resulting in the expression of spike protein that could render the neonatal immune system defenseless to mount an immune response to a subsequent SARS-CoV-2 infection, due to immune tolerance to viral proteins [17].

5. The SARS-CoV-2 Spike Protein Triggers Autoimmune Responses

Autoimmune diseases can be triggered by viral infections and some vaccines, and are more common to females [3]. There is mounting evidence to support the hypothesis that SARS-CoV-2 infection is a risk factor for autoimmune disease in predisposed individuals [95–98]. Autoimmune diseases manifest as hyper-stimulated immune responses against autoantigens, which are normally tolerated by the immune system. The proposed mechanisms of autoimmune response during SARS-CoV-2 infection have been previously discussed [98,99] and include molecular mimicry, bystander activation, epitope spreading, and polyclonal lymphocyte activation by SARS-CoV-2 superantigens. Molecular mimicry describes structural similarities between SARS-CoV-2 antigens and autoantigens that are recognized by immune cells (i.e., cytotoxic T cells) and immunoglobulins (i.e., autoantibodies and antiphospholipid antibodies) in cross-reactive epitopes. When autoantigens are targeted by these effectors, this can lead to immune-mediated tissue damage, and if autoreactive memory B-cells and T-cells are generated, this can lead to chronic disease. Bystander activation involves immune-mediated tissue damage resulting from a nonspe-

cific and over-reactive antiviral innate immune response, such as the cytokine storm that has been described in severely impacted COVID-19 patients. In this case, tissue and cellular components become exposed during damage, and are then ingested by phagocytic cells and presented as autoantigens to autoreactive T helper and cytotoxic T cells, which contribute to ongoing immune-mediated pathology. Epitope spreading refers to ongoing sensitization to autoantigens as the disease progresses, which can lead to progressive and chronic disease. A recent study by Zuo et al. [100] implicated anti-NET antibodies as potential contributors of COVID-19 thromboinflammation; NETs are neutrophil extracellular traps that are produced by hyperactive neutrophils that have either come into contact with SARS-CoV-2 or have been activated by platelets and prothrombotic antibodies. These NETs are cytotoxic to pulmonary endothelial cells, and Zuo et al. discovered that anti-NET antibodies contribute to NET stabilization, which may impair their clearance and exacerbate thromboinflammation. Very recently, NETs were also implicated in VIPIT following the Oxford/AstraZeneca vaccine [101], but the potential involvement of anti-NET antibodies remains to be determined.

SARS-CoV-2 spike protein superantigen activity was discussed earlier. Superantigens are known to trigger the cytokine storm that can lead to immune-mediated multiple organ dysfunction syndrome, and this is often followed by immune suppression that can lead to persistent infection [102]. Superantigens such as SEB have been shown to exacerbate autoimmune disorders (i.e., experimental autoimmune encephalomyelitis and experimental multiple sclerosis) in mice models [103]. Recently, Jacobs proposed that long-COVID could be due in part to SARS-CoV-2 superantigen-mediated immune suppression, leading to persistent systemic SARS-CoV-2 infection [99]. In terms of pregnancy, prenatal exposure of rats to SEB was shown to attenuate the development and function of regulatory T cells in adult offspring [104] and alter the behaviour (i.e., increased anxiety and locomotion) of mice offspring [105].

Among the proposed mechanisms contributing to autoimmune responses during COVID-19, molecular mimicry has recently taken the front stage. A number of studies have found homologies between SARS-CoV-2 amino acid and human protein amino acid residues [106,107], and more specifically, between the spike protein and human proteins [108–110]. Additionally, some of these cross-reactive regions were immunogenic epitopes, meaning that they can bind to MHC I or II molecules on antigen-presenting cells, thereby activating autoreactive B and T cells that elicit an autoimmune response. Martínez et al. [109] for example, identified common host-like motifs in the SARS-CoV-1 and SARS-CoV-2 spike proteins nested in B and T cell epitopes. Morsy and Morsy also identified SARS-CoV-2 spike protein epitopes for MHC I and II molecules that were cross-reactive with the homeobox protein 2.1 (NKX2-1) and ATP-binding cassette sub-family A member 3 (ABCA3) lung proteins [110]. Kanduc and Shoenfeld searched for overlapping SARS-CoV-2 spike protein hexa- and hepta-peptides across mammalian proteomes and found a large number of matches within the human proteome; these authors stated that this is evidence of molecular mimicry, contributing to SARS-CoV-2-associated diseases [108]. Dotan et al. [111] also recently identified 41 immunogenic penta-peptides within the SARS-CoV-2 spike protein that are shared with 27 human proteins related to oogenesis, placentation and/or decidualization, implicating molecular mimicry as a potential contributor to female infertility. Vojdani and Kharrazian also demonstrated that anti-SARS-CoV-2 human IgG monoclonal antibodies cross-reacted with 28 out of 55 human tissue antigens derived from various tissues (i.e., mucosal and blood–brain barrier, thyroid, central nervous system, muscle and connective tissue), and BLAST searches revealed similarities and homologies between the SARS-CoV-2 spike protein and human proteins [112]. In terms of the COVID-19 vaccines, molecular mimicry has also been implicated in myocarditis, an AVR associated with the COVID-19 mRNA vaccines [113]. Huynh et al. [114] also recently identified autoantibodies as the potential cause of VIPIT; these autoantibodies were found to bind to PF4 and allowed for Fc receptor-mediated activation of platelets, which could initiate coagulation, leading to thrombocytopenia and thrombosis. These findings have

raised concerns over the possibility that anti-SARS-CoV-2 spike protein antibodies may be responsible for VIPIT. Greinacher A et al. [115] investigated this hypothesis and found that SARS-CoV-2 spike protein and PF4 share at least one similar epitope. However, when they used purified anti-PF4 antibodies from patients with VIPIT, none of the anti-PF4 antibodies cross-reacted with SARS-CoV-2 spike protein. They therefore concluded that the vaccine-induced immune response against the SARS-CoV-2 spike protein was not the trigger causing VIPIT.

Others have implicated antiphospholipid antibodies in both COVID-19 and VIPIT-related thrombosis. APA are present in 1–5% of healthy people and are associated with the risk of autoimmune antiphospholipid syndrome (APS), which is the most common form of thrombophilia, and is more common in young women. APS during pregnancy is a risk factor for poor maternal and fetal outcomes such as pregnancy-induced hypertension, fetal loss, placental abruption, abortion, thrombosis, preterm delivery, pulmonary embolism, neonatal mortality, fetal growth restriction, premature infants and increased neonatal admission to intensive care units [116]. Antiphospholipid antibodies are a heterogeneous group of autoantibodies that recognize anionic phospholipids and protein–phospholipid aggregates, and are used as a diagnostic biomarker of APS. Bacterial, viral and fungal infections can elicit the production of antiphospholipid antibodies, and molecular mimicry is the proposed mechanism by which this occurs [117]. For example, human β2-glycoprotein I, which contains a highly immunogenic five-domain glycoprotein, displays homology to several microbial peptides [118]. Anti-β2-glycoprotein I antibodies have been detected in COVID-19 patients, and anti-β2-glycoprotein I antibodies are considered to be the most pathogenic antiphospholipid antibodies in APS [117]. β2-glycoprotein I is able to bind to endothelial cells and anti-cardiolipin antibodies, which can result in APA-induced endothelial cell damage [119]. Zussman et al. [119] recently demonstrated that antiphospholipid antibodies were able to bind to placental mitochondria, leading to ROS production. These authors proposed that APA binding to β2-glycoprotein I and cardiolipin in mitochondrial membranes contributes to oxidative stress and placental dysfunction. Antiphospholipid antibodies are also generated in response to vaccination, most commonly reported for influenza vaccines [120]. Martirosyan et al. reviewed cases of paediatric Henoch–Schonlein purpura and lupus associated with influenza vaccines and suggested that long-term effects such as thrombosis could be expected, since antiphospholipid antibodies remained elevated in some lupus patients for at least 6 months post-vaccination [120]. In the case of COVID-19 and COVID-19 vaccine-related AVRs, the role of antiphospholipid antibodies remains controversial, and more data are needed to establish potential cause–effect relationships [121].

Very recently, anti-idiotypic antibodies were also proposed as an autoimmune response following SARS-CoV-2 infection [122]. In this study, Arthur et al. detected ACE2 autoantibodies in convalescent plasma from previously infected patients, which were also correlated with anti-spike protein RBD antibody levels. Since patients with ACE2 autoantibodies also had less plasma ACE2 activity, these authors hypothesized that the ACE2 autoantibodies were anti-idiotypic antibodies that could interfere with ACE2 function and contribute to post-acute sequelae of SARC-CoV-2 infection (PASC, or "long-COVID"). We are unaware of ACE2 autoantibody levels being assessed following COVID-19 vaccination, so this warrants further investigation.

Collectively, the above autoimmune responses triggered by infection with SARS-CoV-2 or the COVID-19 vaccines suggest potential negative outcomes on fetal and neonatal development, and this should be explored in future studies. As with APS, cytokine storms and thromboinflammation are of concern—as is the potential for autoantibody responses that could target fetal/neonatal proteins.

6. The SARS-CoV-2 Spike Protein and Antibody-Dependent Enhancement

While antibodies have a number of important effector activities against SARS-CoV-2, including limiting viral attachment to epithelial cells and viral neutralization, non-

neutralizing antibodies that enhance viral entry into host cells can sometimes also be generated; this immunological phenomenon is referred to as antibody-dependent enhancement (ADE). Since the early days of the COVID-19 pandemic, concerns have been raised about the possibility of ADE occurring, as it has been reported that both SARS-CoV-1 and MERS-CoV infect various animal models via ADE [123,124]. Ricke [124] proposed that SARS-CoV-2 may leverage Fc receptors for host cell invasion, and this may contribute to cytokine storms, leading to adult multi-system inflammatory syndrome, and also infant MIS-C—the latter presumably being mediated by passive transfer of maternal anti-SARS-CoV-2 antibodies that have become bound to Fc receptors on infant mast cells or macrophages [125]. While the potential risk of this type of ADE occurring in response to COVID-19 vaccines remains unknown, experience with SARS-CoV-1 spike protein vaccines demonstrates that it is indeed a possibility which warrants further investigation [124].

A second type of ADE involves non-neutralizing antibodies binding to and then eliciting conformational changes to viral proteins that can lead to enhanced viral adhesion to host cells [123]. Liu et al. [126] recently screened a panel of anti-SARS-CoV-2 spike protein monoclonal antibodies derived from COVID-19 patients and found that some of these antibodies that bind to the N-terminal domain of the spike protein induce open confirmation of the RBD, which enhances the binding capacity of the spike protein to ACE2 and the infectivity of SARS-CoV-2. Interestingly, these infection-enhancing antibodies have been identified in both uninfected and infected blood donors and have been detected at high levels in severe COVID-19 patients; their presence in uninfected people implies that these individuals may be at risk of severe COVID-19 if they later become infected with SARS-CoV-2 [126]. Another recent study has suggested that people may be at risk of infection by the SARS-CoV-2 Delta variant if they were vaccinated against the Wuhan strain spike sequence because the Delta variant is well-recognized by infection-enhancing antibodies targeting the N-terminal domain of the spike protein [127].

A number of murine studies have demonstrated that non-neutralizing maternal antibodies can increase the risk of neonatal disease. For example, pregnant mice infected with different strains of Dengue virus (DENV) display maternal anti-DENV IgG that is passively transferred during gestation and enhances the severity of offspring disease (i.e., hepatocyte vacuolation, vascular leakage, lymphopenia and thrombocytopenia) following infection with the heterotypic strain [128], and breast feeding has been shown to extend the window of ADE [129]. In terms of anti-SARS-CoV-2 antibodies, the passive transfer of anti-SARS-CoV-2 neutralizing antibodies has been detected in milk samples collected from women with COVID-19 [130]; however, non-neutralizing antibodies were not assessed. Anti-spike protein antibodies (IgG and IgA) have also been detected in milk samples from lactating mothers who were vaccinated with SARS-CoV-2 mRNA vaccines [131]; however, their neutralization/non-neutralization status was not assessed. Therefore, we have no data to determine whether or not passive transfer of ADE can occur during SARS-CoV-2 infection or COVID-19 vaccination, and so this warrants further investigation.

7. Using an Ovine Model to Study Long-Term Outcomes of Maternal COVID-19 Vaccination

Assessing long-term outcomes of vaccination in pregnant women and their offspring under the DOHaD paradigm is difficult. Humans have a long-life span and a generation interval of between 26–30 years. Differences in gender and genetics contribute to variations in immune responses, and environmental factors such as socioeconomic status, comorbidities, diet, exercise and vices act as interacting variables.

Large animal models such as sheep offer numerous advantages for DOHaD research. Sheep have a generation interval < 1.5 years, a long gestation period of 145 days, and their fetal size and rate of development is comparable to humans [132]. The ovine respiratory and cardiovascular systems are physiologically similar to humans, which make them an ideal model for studying respiratory diseases, neonatal lung development and xenotransplantation [133]. Pregnant sheep have been used to study connections between maternal allergies and offspring lung development and risk of allergies [134,135]. Sheep are also

considered ideal models for studying neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases [136], and due to their susceptibility to scrapie, are widely used to study prion diseases [137,138]. Sheep are also a well-recognized biomedical model for studying immunology [139], vaccine development and safety [140,141], and have been extensively used by our group and others to study the impact of maternal inflammation during pregnancy on offspring development [142]. We have shown, for example, that transient inflammation lasting approximately 6 h, elicited by an immune challenge with *Escherichia coli* LPS endotoxin at gestation day 135, is sufficient to alter male and female offspring immune responsiveness at 4.5 months of age [143], and neuroendocrine responsiveness at 5.5 months of age [144,145]. Using this endotoxin model, we have explored candidate protein and miRNA biomarkers for assessing the acute-phase response (APR) to immune challenge [146,147], and characterized variations in the APR at the population level [148], showing a moderately heritable phenotype [149].

As this pandemic continues to evolve, there are many unknowns with regards to COVID-19 and DOHaD. For example, what will be the outcomes of newborn babies born to mothers who have COVID-19 during pregnancy? [150]. Furthermore, what will be the outcome of offspring from mothers who received COVID-19 vaccines [151], and will this depend on gestational stage of exposure? With recent evidence of decreased vaccine efficacy against new SARS-CoV-2 variants [152], and immunity waning over time [153], addressing vaccine safety during pregnancy is becoming increasingly important, as efforts are already underway in some countries to administer more booster immunizations. Since epigenetic mechanisms are believed to contribute to the risk of DOHaD [154], a systems biology approach to addressing these questions is warranted, and we believe that the ovine model offers unique advantages for assessing the long-term impact of maternal COVID-19 vaccination on offspring health. Spike protein biodistribution studies can be performed during pregnancy and lactation by harvesting various tissues after euthanasia, and vaccine protocols can be mixed (i.e., primary immunization with Pfizer–BioNTech followed by booster immunization with Moderna) to assess the efficacy and safety of mixed vaccines. Offspring health can be assessed in terms of neonatal growth, gut development and neuroendocrine–immune system function. If phenotypic changes are observed, then mRNA, microRNA and circularRNA sequencing can be performed using different tissues to better understand any potential epigenetic changes brought on by maternal vaccination. Since there is evidence that the SARS-CoV-2 spike protein is capable of binding to ovine ACE2 [155], and that ovine respiratory organ cultures are susceptible to SARS-CoV-2 infection [156], this model species could also be used to investigate the potential impact of molecular mimicry and ADE on neonatal health.

8. Conclusions

In closing, we currently have no data to assess the outcome of maternal COVID-19 vaccination on offspring health, and this may take years to generate. We believe that the ovine model can be used to rapidly assess potential concerns about the administration of COVID-19 vaccines during pregnancy, and that the knowledge gained will help us to predict potential health outcomes in human offspring, which could lead to the development of treatments to help mitigate any potential adverse outcomes.

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This is Exhibit W referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

nutrients

MDPI

Review

Immunoceuticals: Harnessing Their Immunomodulatory Potential to Promote Health and Wellness

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Abstract: Knowledge that certain nutraceuticals can modulate the immune system is not new. These naturally occurring compounds are known as immunoceuticals, which is a novel term that refers to products and systems that naturally improve an individual's immuno-competence. Examples of immunoceuticals include vitamin D3, mushroom glycans, flavonols, quercetin, omega-3 fatty acids, carotenoids, and micronutrients (e.g., zinc and selenium), to name a few. The immune system is a complex and highly intricate system comprising molecules, cells, tissues, and organs that are regulated by many different genetic and environmental factors. There are instances, such as pathological conditions, in which a normal immune response is suboptimal or inappropriate and thus augmentation or tuning of the immune response by immunoceuticals may be desired. With infectious diseases, cancers, autoimmune disorders, inflammatory conditions, and allergies on the rise in both humans and animals, the importance of the use of immunoceuticals to prevent, treat, or augment the treatment of these conditions is becoming more evident as a natural and often economical approach to support wellness. The global nutraceuticals market, which includes immunoceuticals, is a multi-billion-dollar industry, with a market size value of USD 454.55 billion in 2021, which is expected to reach USD 991.09 billion by 2030. This review will provide an overview of the immune system, the importance of immunomodulation, and defining and testing for immunocompetence, followed by a discussion of several key immunoceuticals with clinically proven and evidence-based immunomodulatory properties.

Keywords: immunoceuticals; nutraceuticals; immunomodulation; immunocompetency

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1. Introduction

The term "immunoceuticals" cannot be defined without first defining the term "nutraceuticals", which was coined by Stephen L. DeFelice in 1989 as a blend of the two words "nutrition" and "pharmaceuticals" [1]. Despite the term nutraceuticals being first defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease", there is currently no internationally standardized definition for "nutraceuticals", and many have tailored or fine-tuned its definition overtime [1,2]. For example, Health Canada defined a nutraceutical as "a product isolated or purified from foods that is generally sold in medicinal forms not usually associated with food [3]. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease". For the purposes of this review, nutraceuticals are natural products that not only provide nutritional value but also help to support general health. When consumed above a threshold concentration, these natural products may have beneficial pharmacological effects. Therefore, the term nutraceuticals will be used to highlight the therapeutic values of certain natural products that are known to play a role in health performance

beyond the nutritional value that they provide. Examples of nutraceuticals include, but are not limited to, certain vitamins, minerals, fatty acids, amino acids, peptides, proteins, and pre/probiotics.

"Immunoceuticals", like nutraceuticals, is a portmanteau word that blends "immunity" and "pharmaceutical". Therefore, any nutraceuticals that demonstrate beneficial immunomodulatory mechanisms that support an optimal immune system and/or modify immunological status to defend against various diseases such as cancers and infectious or autoimmune diseases can be separately categorized as immunoceuticals (Figure 1). Based on this definition, examples of immunoceuticals would include vitamin D3, mushroom-derived polysaccharides, plant-derived ergosterols, flavonols, terpenoids, carotenoids, aloe-associated polysaccharides, quercetin, omega-3 fatty acids, and micronutrients such as zinc and selenium, to name a few. Immunoceuticals are structurally different compounds with different modes of action. However, they all work to optimize immunological functions.

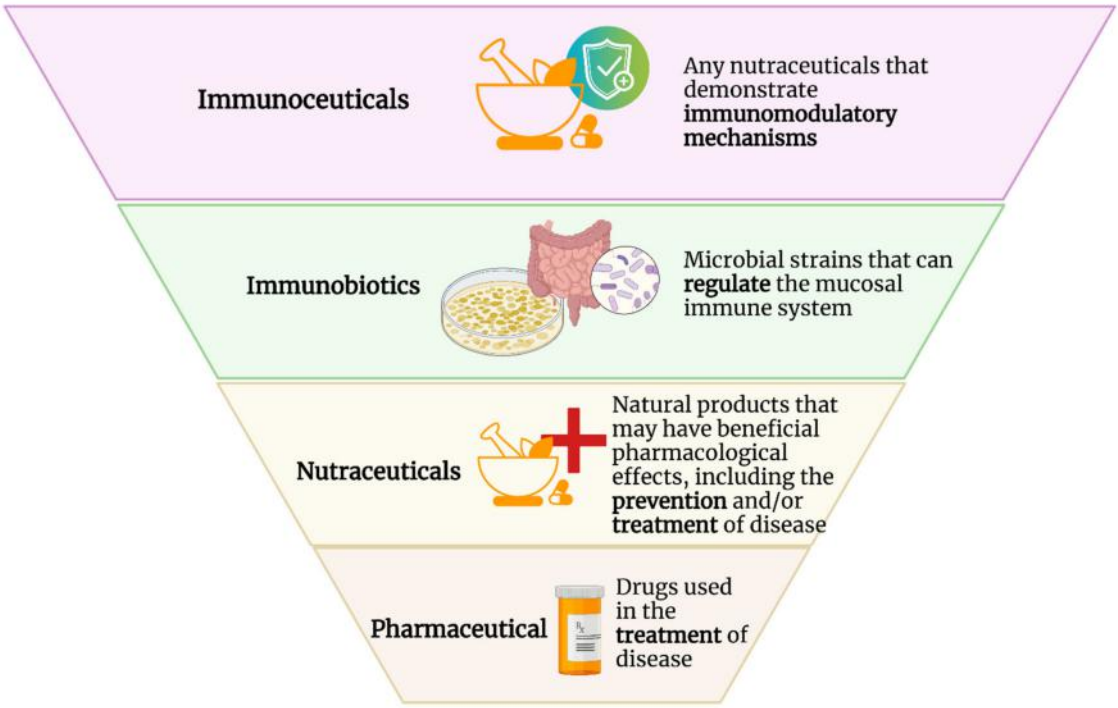

Figure 1. Comparison of the terms "pharmaceutical", "nutraceutical", "immunobiotics", and "immunoceuticals".

1.1. Brief Overview of the Immune System and Its Regulation

The immune system protects against invading pathogens such as bacteria, viruses, fungi, and parasites, as well as cancers, by distinguishing dangerous foreign and modified self-antigens from self-antigens from non-dangerous self and targets them for elimination using a diverse set of host defense mechanisms [4,5]. The immune system is composed of a collection of cells, molecules, tissues, and organs and contains two main branches: the innate and adaptive immune systems. Innate host defence, sometimes referred to as non-specific immunity provides the first line of defence using a wide array of cells and molecules against an invading pathogen. It is non-specific in that the innate defence mechanisms use a limited array of receptors to respond to dangerous molecules, and each response is mounted as if seeing them for the first time or with only transient forms of memory training. These mechanisms have broad specificity and are fast-acting, with responses initiated within seconds to hours.

The adaptive immune response can be further divided into two categories: antibody and cellular responses mediated by B- and T-lymphocytes, respectively. Adaptive immune

responses are differentiated from innate responses in that they are antigen-dependent, clonally antigen-specific, and possess immunological memory that can last years, or even a lifetime. Due to its exquisite antigen specificity and durability, the adaptive immune defences, particularly upon initial exposure, require several days to two weeks to mount a response. However, as a result of immunological memory, subsequent exposure to the same antigen allows for a far more rapid and higher magnitude immune response that can usually eliminate an infection prior to the onset of the disease. It is also important to understand that the innate and adaptive immune systems are not independent, but rather communicate to provide a carefully orchestrated collection of defence mechanisms shaped by genetics of the host and the nature of the encountered invader.

When performing optimally, the innate and adaptive responses provide immunity, which means protection or exemption from disease, ideally accompanied by an inability to transmit the causative agent to others. Protection may be provided passively—for example, antibodies delivered to a newborn via the mother's first milk, known as colostrum—or actively via naturally acquired immunity and artificially induced immunity. Naturally acquired immunity is commonly achieved following infection and recovery from exposure to a pathogen. Artificially acquired immunity may be achieved through vaccination, which attempts to recapitulate the gold-standard of naturally acquired immunity. However, it is critical to understand that not all immune responses are optimal or protective. The immune system is often referred to as a double-edged sword, where protection is carefully balanced against immuno-pathology or damage caused by prolonged overt or inappropriate immune responses. Thus, a critical role of immunoceuticals lies in helping to tune optimal host defence.

The regulatory mechanisms of the immune system are vast and complex, with many different regulators and factors involved that affect how the immune system functions and responds. It is widely recognized that the manifestation of infection varies greatly from mild to severe among individuals, and this is due, in part, to the genetics of both the host and the pathogen. In fact, around 5000 of the 23,000 genes in mammals are dedicated to host defence [6]. This makes sense, given that the immune system governs survival. However, adaptive changes induced by environmental influences, especially during early life development, also play a large role in helping to shape an individual's immune system [7]. The interplay between genes and the environment is commonly referred to as gene-by-environmental interactions, and epigenetic mechanisms govern these. For example, environmental pathogen exposure helps shape the adaptive immune system repertoire in terms of what T- and B-cell clones predominate in a given host. The biology of the pathogen also plays a role; for example, its virulence, the portal of entry and the dose of exposure to the pathogen all affect disease outcome. Collectively, the host–pathogen–environment triad shapes disease consequences.

Epigenetic modifications are changes to DNA that do not involve alterations to the genomic sequence; typically involving DNA methylation, histone modifications, or small noncoding RNAs. These changes are induced by environmental stimuli, including but not limited to exposure to immunomodulatory immunoceuticals, such as colostrum [8], probiotics [9], quercetin [10], kaempferol [11], and curcumin [12]. Epigenetic modifications can occur in cells of the immune system and can be passed on with each cell division to daughter cells. Consequently, epigenetic modifications can potentially influence immunological phenotypes and impact disease outcomes. In some cases, these changes are passed on to future generations. For example, global and gene-specific DNA methylation and alterations in fatty acid content by arachidonic acid exposure are maintained across generations, which may prove beneficial to the innate immune system [13]. Additionally, the epigenetic effects of preconceptual paternal infection and activation of paternal immune responses can affect offspring phenotypes, particularly brain function, behavior and immunological functions across multiple generations without re-infection [14].

There are a variety of cells, such as regulatory T cells and dendritic cells, and molecules, such as transcription factors (e.g., nuclear factor-kB), cytokines, and microRNAs, that serve

as key regulators of the immune system. Working in concert, this complex communication network ensures that the immune system is functioning effectively to distinguish between the normal self and the dangerous non-self and to ensure that the response is not excessive and potentially damaging to tissues. Continuous cross-talk between the innate and adaptive immune system, the immune system and the hypothalamic–pituitary–adrenal axis, the gut–brain connection, and the mucosal and central immune system are also important aspects of the regulation of the immune response. Cross-talk between innate and adaptive responses plays a key role in the regulation and appropriate activation of the adaptive immune system. For example, the activation of adaptive immunity is dependent on a subset of innate leukocytes called antigen-presenting cells, which activate helper T cells, which then go on to modulate the cellular and humoral immune responses of the adaptive immune system, which can also involve elements of the innate immune system (e.g., macrophages) [15]. Communication between the hypothalamic–pituitary–adrenal axis and the immune system, the gut–brain connection, as well as the common mucosal immune system can influence and regulate the function of leukocytes via the production of hormones secreted by the pituitary, adrenal, and other endocrine organs, as well as serving as an ideal environment to educate meningeal B cells to produce IgA antibodies against specific microbes, such as those causing meningitis [16,17]. The details of these connections are outside the scope of this review.

1.2. Inflammation and Immunopathology

Inflammation is an integral component of the immune system and is a protective strategy that is designed to alert the body to danger and recruit leukocytes to the site of infection or tissue trauma. Classical signs and symptoms of inflammation include heat, fever, redness, swelling, and pain, the combination of which can often lead to a loss of function in the affected tissue or organ. The molecular mechanism of inflammation is a complicated process that is tightly regulated by several key regulators, including inflammasomes [18], inflammatory caspases (e.g., caspases −1, −4, −5, −11, and −12) [19], and pro-resolving lipid mediators [20] to name a few. Cellular homeostasis must subsequently be restored after the harmful stimuli has been terminated to promote the healing of damaged tissues. However, chronic inflammation can occur if there is a failure in eliminating the noxious stimuli [21]. Genetic defects in the innate or adaptive host defence mechanisms can also result in the development of chronic inflammatory diseases, among other immunopathological disorders including autoimmunity, immunodeficiency, and hypersensitivity reactions (e.g., allergic reactions). Poorly regulated inflammatory responses and tissue damage resulting from inflammation are key immunopathological features [5]. Immunosenescence is another contributing factor due to an age-associated decline in immune responses, which contributes to increased risk of infectious and inflammatory diseases [22].

The importance of addressing chronic inflammation cannot be overstated, as inflammation plays a primary role in the etiology of many diseases including atherosclerosis, obesity, type 2 diabetes, asthma, inflammatory bowel disease, neurodegenerative diseases, rheumatoid arthritis, psoriasis and cancer [21]. Furthermore, our understanding of the pathological processes of chronic inflammatory diseases is relatively limited [21]. Developing safe and effective anti-inflammatory therapeutic interventions, including immunoceuticals, remains a crucial goal.

1.3. The Importance of Immunomodulation

Immunomodulation refers to all therapeutic interventions intended to beneficially modify the immune response [23]. Pathological conditions characterized by insufficient immunological function (immunodeficiency), or situations in which a normal immune response is suboptimal, overly aggressive, or inappropriate in controlling an infection or pathological condition, are circumstances in which augmentation or tuning of an immune response is desirable. Augmentation of the immune response in states of immunodeficiency is beneficial in preventing infection and fighting established infections and cancers [23].

Immunodeficiency can develop as a consequence of malnutrition, treatment of cancers (e.g., chemotherapy and radiation), viral infections (e.g., human immunodeficiency virus), and genetic defects or genetic predispositions [23,24].

Heritable and non-heritable factors in shaping human immune systems have been reported. Genetic variation is an important driver of immune variation, and factors such as age, sex, diet, environmental exposure, and microbiome are demonstrated to affect immune responses [25]. It has been well-established in animal models that individuals can be classified based on their ability to mount robust and balanced immune responses, into high, average or low immune responders [24,26,27]. The high immune responders are significantly less likely to become sick compared to the others and are, therefore, less likely to require therapeutic interventions [24,26]. Conversely, the average and low responders have a greater need for the benefits conferred by immunoceuticals which help to enhance their ability to make protective responses. The immune system is controlled by many genes whose expression levels differ from individual to individual, and this determines their propensity to mount protective immune responses following exposure to a pathogen. This is one reason why a diverse set of signs and disease outcomes are observed among individuals exposed to the same pathogen. There are also circumstances in which immunomodulation to attenuate a harmful immune response is desirable, and these circumstances include allergies and autoimmune diseases [23]. The potential to modify undesirable immune responses is a growing area of interest with vast potential to improve health. Genetic defects causing immunodeficiencies may require other interventions and are not within the scope of this review.

1.4. Traditional Immunomodulators

Traditionally, immunosuppressive drugs, immunomodulatory corticosteroids, vaccines, and antibiotics have been used to either augment immunological functions, or to suppress pathological immune responses. However, administration of these compounds can also be accompanied with negative consequences such as unwanted side effects, drug interactions and resistance, adverse events following vaccination, or antimicrobial resistance attributed to overuse of antibiotics. Additionally, most of these drugs end up in the urine and feces with poorly defined environmental consequences [28]. Furthermore, according to the United States Food and Drug Administration (FDA), up to 90% of all experimental drug compounds that go through clinical trials fail to gain FDA approval due to issues with efficacy, formulation, pharmacokinetics, toxicology, or clinical safety [29].

1.5. Clinical Benefits of Immunoceuticals

The importance of immunoceuticals to prevent, treat or augment treatment of pathological conditions such as cancers, viral and bacterial infections, chronic inflammatory diseases, autoimmune disorders and allergies is becoming more evident as a natural approach to support wellness [30]. In Figure 2, the potential utility of immunoceuticals is demonstrated by using infection with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) as an example, and/or adverse events following receipt of a vaccine intended to protect against.

For instance, the novel coronavirus disease that was first identified in 2019 (COVID-19). In Table 1, the applications of immunoceuticals are demonstrated in terms of treating respiratory and gastrointestinal tract infections, cancers, and acquired immunodeficiency syndrome (AIDS).

Immunoceuticals, as a specific subcategory of nutraceuticals, offer a focused approach of supporting the immune system and ensuring its optimal functioning. Many immunoceuticals could be made readily available with revised guidelines that focus on optimizing their immunomodulatory potential to facilitate minimizing disease burdens at the population level. One example is supplementing with higher concentrations of vitamin D than that traditionally recommended in nutritional guidelines.

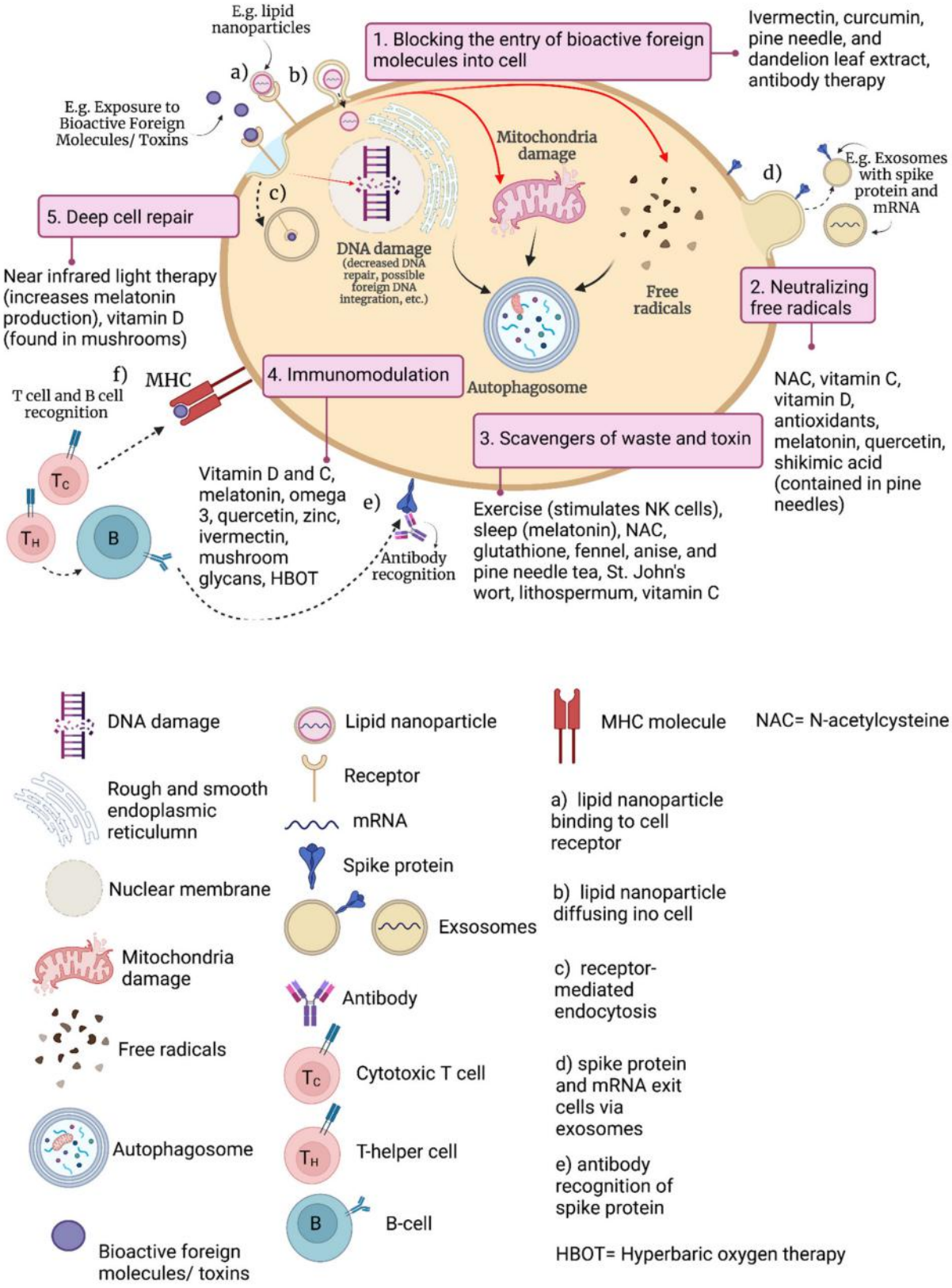

Figure 2. Ways to naturally optimize and regulate the immune system (e.g., following infection with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), or an adverse event following vaccination against SARS-CoV-2, both resulting in exposure to spike protein). MHC = major histocompatibility complex.

Table 1. Clinically proven immunoceuticals for treating respiratory and/or gastrointestinal tract infections, cancers, and acquired immunodeficiency syndrome (AIDS).

Immunoceuticals	Authors	Study Design	Disease/Pathological Condition Addressed with Immunoceuticals	Dose	Results
Vitamin D3 (Cholecalciferol)	[31]	Multi-center, randomized clinical trial	Severe acute respiratory syndrome- coronavirus-2 (SARS-CoV-2) infection	5000 IU or 1000 IU of Vit D3 once daily for two weeks	• Vit D supplementation significantly increased serum 25(OH)D levels in the 5000 international units (IU) group • 5000 IU of daily Vit D3 supplementation reduced recovery time for cough and gustatory sensory loss in patients with mild to moderate COVID-19
	[32]	Quasi-experimental study	SARS-CoV-2 infection	Oral bolus of 80,000 IU Vit D3 during or just before infection with COVID-19	• 82.5% of participants in intervention group survived infection with COVID-19 versus 44.4% in comparator group • Intervention group had longer survival time than Comparator group (log-rank $p = 0.002$) • Vit D3 supplementation inversely associated with Ordinal Scale for Clinical Improvement score for COVID-19 ($\beta = -3.84$ (95% CI:−6.07;−1.62), $p = 0.001$)
	[33]	Randomized clinical trial	SARS-CoV-2 infection	Oral supplementation of 10,000 IU daily of Vit D3 for 14 days	• 10,000 IU of Vit D3 daily for 14 days was sufficient to raise Vit D concentrations • supplemented group presented fewer symptoms than non-supplemented on day seven and fourteen of follow-up
	[34]	Multicenter, randomized, double-blind, placebo-controlled, parallel-group trial	Influenza A infection	1200 IU of Vit D3 daily	• Vit D3 supplementation during winter season may reduce incidence of influenza A-mediated illness • Influenza A infections occurred in 18/167 children in the Vit D3 group versus 31/167 children in the placebo group

Table 1. *Cont.*

Immunoceuticals	Authors	Study Design	Disease/Pathological Condition Addressed with Immunoceuticals	Dose	Results
Polysaccharide K (PSK)	[35]	Randomized double-blind trial	Colorectal cancer	3 g/day starting 10–15 days after surgery until two months after surgery, then 2 g daily until 24 months and 1 g daily thereafter	• rate of patients in remission was significantly higher in PSK group versus placebo group • Survival rate in PSK group significantly higher ($p < 0.05$) than control group • Polymorphonuclear leukocyte activities in PSK-treated patients significantly enhanced
	[36]	Randomized, controlled trial	Colorectal cancer	3 g of PSK per day for over three years	• Disease-free and overall survival of PSK group were longer than those of the control group
Polysaccharide-Peptides (PSP)	[37]	Double-blind placebo-controlled randomized study	Non-small cell lung cancer (NSCLC)	340 mg of purified Yun-zhi PSP capsules three times daily for 28 days	• significant improvement in blood leukocyte and neutrophil counts, serum IgG and IgM, and % of body fat in PSP group, but not control ($p < 0.05$) • 5.9% of PSP patients were withdrawn due to disease progression versus 23.5% of control patients • PSP treatment associated with slower deterioration in advanced NSCLC patients
Probiotics	[38]	Randomized controlled open-label trial	Acute upper respiratory tract infections (acute URTI)	300 mL/day of yogurt supplemented with a probiotic strain, *Lactobacillus paracasei* N1115 (N1115), 3.6×10^7 CFU/mL for 12 weeks	• number of persons diagnosed with acute URTI and number of URTI events significantly decreased in probiotic group versus control • Percentage of $CD3^+$ cells in intervention group significantly higher than in control
Prebiotic and probiotic	[39]	Community based double-masked, randomized controlled trial	Diarrhea, respiratory infections and severe illnesses in children aged 1–4 years of age	milk fortified with 2.4 g/day of prebiotic oligosaccharide and 1.9×10^7 CFU of probiotic *Bifidobacterium lactis* HN019	• incidence of dysentery episodes, pneumonia and severe acute lower respiratory infection reduced by 21%, 24%, and 35%, respectively

Table 1. *Cont.*

Immunoceuticals	Authors	Study Design	Disease/Pathological Condition Addressed with Immunoceuticals	Dose	Results
Quercetin	[40]	Prospective, randomized, controlled, and open-label study	SARS-CoV-2 infection	1000 mg of Quercetin/day for 30 days	• statistical improvement of all clinical outcomes (need and length of hospitalization, need of non-invasive oxygen therapy, progression to Intensive care unit, and number of deaths) • 1000 mg of Quercetin/day was well tolerated by all subjects
	[41]	Second, pilot, randomized, controlled and open-label clinical trial	SARS-CoV-2 infection	600 mg of Quercetin/day for seven days, followed by 400 mg of Quercetin/day for another seven days	• 16 of the 21 COVID-19 outpatients in the Quercetin group tested negative for SARS-CoV-2, and 12 patients in the Quercetin group had all their symptoms diminished one-week post-treatment • Quercetin significantly improved virus clearance, symptom frequency, lactate dehydrogenase, and ferritin
Beta-Carotene (Carotenoid)	[42]	Pilot study	AIDS	60 mg/day for four weeks	• Total lymphocyte counts increased by 66% and CD4+ cells rose slightly • Patients with a baseline CD4+ cells greater than 10/ul demonstrated an average increase of 53 $\pm$ 10 cells/ul
Omega-3 fatty acids	[43]	Single-blind randomized controlled trial	SARS-CoV-2 infection	2 g of docosahexaenoic acid (DHA) + eicosapentaenoic acid (EPA) for 2 weeks	• significantly decreased fatigue and body pain, and increased appetite in intervention group • decreased erythrocyte sedimentation rate and C-reactive protein following two weeks of omega-3 supplementation
	[44]	Double-blind, randomized clinical trial	SARS-CoV-2 infection	One capsule of 1000 mg omega-3 daily containing 400 mg EPAs and 200 mg DHAs for 14 days	• significantly higher one-month survival rate and higher arterial pH, HCO_3, and base excess (respiratory function parameters) and lower blood urea nitrogen, creatinine, and potassium (renal function parameters) in intervention group versus control group (both composed of critically ill COVID-19 patients)

Table 1. *Cont.*

Immunoceuticals	Authors	Study Design	Disease/Pathological Condition Addressed with Immunoceuticals	Dose	Results
Melatonin	[45]	Single-center, double-blind, randomized clinical trial	SARS-CoV-2 infection	3 mg of melatonin three times daily for 14 days	• Significant improvement in clinical signs and symptoms (cough, dyspnea and fatigue), as well as C-reactive protein concentrations and pulmonary involvement in intervention versus control • Significantly shorter mean time of hospital discharge and return to baseline health in intervention versus control

COVID-19 = Novel coronavirus disease identified in 2019; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2; I.U. = international units; PSK = polysaccharide K; PSP = polysaccharide-peptides; Vit D3 = vitamin D3/cholecalciferol; CFU = colony forming units; URTI = upper respiratory tract infection; CD = cluster of differentiation; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; NSCLC = non-small cell lung cancer; IgG = immunoglobulin G; IgM = immunoglobulin M; $HC0_3$ = bicarbonate.

1.6. Economic Considerations for Nutraceuticals

The global nutraceutical industry had a market size value of $454.55 billion USD in 2021. This is expected to reach $991.09 billion USD by 2030, expanding at an expected compound annual growth rate of 9.0% from 2021 to 2030 [46]. From the perspective of consumers, many immunoceuticals are available at relatively economical prices compared to pharmaceuticals. This is because, immunoceuticals are easily accessible as they are natural products generally found in foods or in the case of vitamin D by appropriate sun exposure. They are also supported by a wide breadth of scientific literature and clinical studies to support their safety, efficacy, and immunomodulatory properties [47].

1.7. Defining and Testing for Immunocompetence

Immunocompetence is defined as the ability of an organism's immune system to elicit an appropriate immune response following exposure to an antigen [48]. Protection against pathogenic processes requires proper functioning immune system, which includes surveillance for antigens and the elicitation of a rapid and effective response following exposure that is resolved as quickly as possible. Since disturbances in immune functionality can affect immunocompetence thereby increasing an individual's susceptibility to infection and disease, there is merit in measuring various immunological parameters as a way of assessing an individual's immunocompetence.

Measuring leukocyte function and numbers is a way that immunocompetence can be assessed. Cells of the immune system are collectively referred to as leukocytes, which can be further divided into lymphocytes, monocytes, and granulocytes. Due to the diverse functional differences among these cells and their subsets, leukocyte function can be assessed using a number of functional assays [48,49]. Since not all leukocytes possess the ability to proliferate in response to antigen, migrate to the site of infection, possess phagocytic and cytotoxic properties, or generate antibodies, interferons and interleukins, particular assays are required to assess distinct cell functions. While many immune assays are performed *in vitro*, *in vivo* tests are also available and provide a more holistic assessment of immunocompetence. Examples of in vivo measures include the delayed-type hypersensitivity skin test, which is based on the reaction that occurs following an intradermal injection of an antigen and is an indicator of T cell responsiveness, or the concentration of antibodies produced following the administration of a vaccine or test antigen. Quantitative measures of the numbers of leukocytes and their products can also be used to assess immunocompetence instead of assessing cell activity. Total white blood cell counts (WBC) in humans only provide a crude quantitative measure of immunocompetence as the normal range for WBC values is extremely variable (5000–10,000 cells/mm^3) [48]. Thus, deviations from the normal range can be difficult to interpret without a differential analysis of the absolute and relative numbers of lymphocytes and their subpopulations, monocytes, and granulocytes. Another quantitative assessment of immunocompetency includes assessing antibody status. For example, elevated concentrations of autoantibodies can be suggestive of autoimmune disease, whereas elevations of antibodies of the appropriate isotype against most pathogens may be indicative of protection, but antibodies against latent viruses such as the herpes simplex virus or Epstein-Barr virus may indicate reinfection or reactivation of the virus, and therefore, decreased immunocompetency [50]. As such, it is critical to understand the nature of the immune response being evaluated in order to provide accurate interpretation prior to making therapeutic recommendations.

1.8. Nutritional Immunology

Individual nutritional status is an important predictor of the health and effectiveness of a host's immune system because the immune response against a pathogen, cancer or toxin is metabolically costly.

Undernutrition or imbalanced nutrition (malnutrition) are positively correlated with immunodeficiency, with almost all immunological effector mechanisms being affected, especially the non-specific defences and cell-mediated immunity [51]. Undernutrition

has a deteriorative effect on immune responses, eventually resulting in the atrophy and dysfunction of immunological organs and tissues, and changes in the numbers, ratios, and functions of different leukocyte immunophenotypes [51]. Likewise, protein and energy malnutrition has been linked to destruction of cell-mediated immunity, phagocyte function and the complement system, and decreased antibody concentrations, especially secretory immunoglobulin A, and/or production of cytokines [52].

The past few decades have led to an increased understanding of how naturally occurring compounds, such as the immunoceuticals abscisic acid and conjugated linoleic acid; *n*-3 fatty acids; and vitamins A, D, and E can modulate immune responses. Moreover, the nutritional value that these naturally occurring compounds provide in the diet, they can also dynamically influence the immune system [51]. More than 65% of leukocytes in the body are found in the gut, thus making the gut one of the largest immunological organs [51]. Additionally, the innate immune receptors in the gut serve as primary targets for immunomodulation via the diet.

The key to preventing immunodeficiencies resulting from malnutrition or nutritional deficiencies is provision of whole and balanced nutrition starting right in the womb. Nutritional care is of utmost importance during pregnancy, infancy and childhood, and in old age, as these demographics are more vulnerable and have either an immune response bias, or an immature or aging immune system, respectively, that does not function at its optimal potential. A cost -effective approach to minimizing immunodeficiencies related to nutrition is to fortify foods with nutrients such as vitamin D, A, E, zinc and selenium, so they can be obtained without requiring changes to dietary habits, particularly when those lifestyle changes are not obtainable in certain scenarios [51]. Examples of foods that are fortified with such nutrients include cereals and cereal products, milk and milk products, fats and oils, beverages and infant formulas. Biofortification, such as the addition of probiotics, is another way in which foods can be fortified and act as immunoceuticals [53].

Micronutrient deficiencies in developed countries leading to immune system problems are mainly caused from dietary restriction (cultural or religious or personal habits), which may lead to the selection of certain foods and not others [51]. Therefore, consumption of a diverse diet is required to avoid nutritional deficiencies. Severe deficiency of nutrients (micro or macro) may require supplementation of nutrients along with a normal diet to improve immunity.

It is important to note that the supplementation of specific nutrients is recommended in situations of deficiency, and not in situations of sufficiency. Nutritional deficiency is defined as an inadequate supply of essential nutrients in the diet leading to malnutrition and/or disease [54]. In contrast, nutritional sufficiency is defined as an adequate supply of essential nutrients in the diet, thus preventing malnutrition and the development of diseases related to malnutrition. Supplementation of specific nutrients in situations of nutritional sufficiency may not always be beneficial and may cause harm. For example, supplementation of iron under disease challenge may not be beneficial as many pathogens rely on the bioavailability of iron to proliferate [55]. As such, host defence strategies have the ability to limit the availability of iron for bacteria by sequestering iron away [55]. Thus, the value of knowledge across immunological disciplines when dealing with immunoceuticals, including developmental and nutritional immunology and immunotoxicity, is of utmost importance. Certain immunoceuticals when not properly formulated, or when consumed at an inappropriate concentration can lead to immune system imbalances resulting in inflammation or immunosuppression.

2. Key Immunoceuticals and Their Immunomodulatory Properties

The immunomodulatory properties of several immunoceuticals are summarized in Table 2. The following sections will highlight a few of these key immunoceuticals.

Table 2. Immunomodulatory properties of immunoceuticals.

Immunoceutical	Immunomodulatory Properties
Vitamin D3 (Cholecalciferol)	• Regulates production of antimicrobial peptides (cathelicidin and defensin); ↑ expression of antimicrobial peptides [56] • ↑ expression of proteins involved in intercellular connections (connexin-43, tight junctions, and E-cadherin) in epithelial barriers [56] • Vitamin D receptor (VDR) expressed in almost all leukocytes (i.e., activated $CD4^+$ and $CD8^+$ T cells, B cells, and antigen-presenting cells, such as macrophages and dendritic cells); receptor-ligand pair (Vit D3 and VDR) acts as a strong immunosuppressor [56,57] • Enhances mobility and phagocytosis of macrophages, and ↑ generation of tumor necrosis factor (TNF)-$\propto$ by macrophages [58] • Causes neutrophils to traffic to sites of inflammation and stimulates them to kill microbes [56] • Controls interferon (IFN) production [56] • Inhibits the proliferation, differentiation, and production of antibodies by B cells [56] • Inhibits differentiation and maturation of dendritic cells, ↓ expression of major histocompatibility complex (MHC)-II and auxiliary stimulative molecules such as B7 and CD40 on dendritic cells, and thus, ↓ cytotoxicity of $CD8^+$ T cells [56] • Reduces T lymphocyte proliferation and regulates skewing towards particular $CD4^+$ T cell subsets [57] • Shifts cytokine patterns from a Th-1 to a Th-2 milieu by inhibiting cytokines required for Th1 differentiation (e.g., IL-12) or produced by differentiated Th1 cells (e.g., IL-2 and IFN-γ), and augmenting Th2 cell development to promote self-tolerance [57,59] • Activates renin-angiotensin system (RAS) pathway by inducing transforming growth factor (TGF)-β-1 [56] • Reduces risk of developing autoimmune diseases (e.g., Type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, Crohn's disease, thyroiditis, psoriasis, polymyalgia rheumatic, autoimmune gastritis, and systemic sclerosis) [60,61]

Table 2. *Cont.*

Immunoceutical	Immunomodulatory Properties
Coriolus versicolor extract (polysaccharide krestin (PSK), polysaccharide peptide (PSP))	• PSP induces expression of TNF-∝; pro-inflammatory cytokine with potent tumoricidal activity and capable of inducing apoptosis [62] • PSP increases production of IL-12; Th1-related cytokine that enhances the cytotoxic activities of natural killer and $CD8^+$ T cells and their expression of TNF-∝ [62] • PSP induces interleukin (IL)-1β; a pleiotropic cytokine and pro-inflammatory signal to enhance lymphocyte proliferation and differentiation [62,63] • Coriolus versicolor (CV) extract activates T lymphocytes, B lymphocytes, monocytes/macrophages, bone marrow cells, natural killer cells and lymphocyte activated killer cells in vitro [64] • Promotes the proliferation and/or production of antibodies and various cytokines (i.e., IL-2 and IL-6, IFNs, and TNF-∝) [64] • CV extracts shown to restore certain depressed immunological responses caused by tumor burden or chemotherapy treatment to normal levels [65–67]
Quercetin	• Neutralizes free radicals through the donation of hydrogen atoms; antioxidant activity increases cell survival rate [68] • Strong reducing agent; provides protection against oxidative stress [69] • Inhibits production of cyclooxygenase (COX) and lipoxygenase (LOX) inflammatory enzymes [70] • Induces gene expression and production of Th-1 derived IFN-γ, and down-regulates Th-2-derived IL-4 by blood mononuclear cells [70] • Direct regulatory effect on basic functional properties of leukocytes via the extracellular regulated kinase 2 (Erk2) mitogen-activated protein kinase (MAPK) signaling pathway in human mitogen-activated blood mononuclear cells and purified T lymphocytes [70]
Carotenoids	• Strong antioxidant agents to combat oxidative stress caused by cytokine storm induced by the innate immune system in response to viral infections [71] • Vitamin C improves pulmonary function and decreases risk of acute respiratory distress syndrome [71] • Vitamin E alleviates oxidative damage and inflammation induced by SARS-CoV-2 [71] • β-carotene and lycopene possess anti-inflammatory properties due to their reactive oxygen species (ROS)-scavenging activities [71]

Table 2. *Cont.*

Immunoceutical	Immunomodulatory Properties
Probiotics	• Activates naïve T and B cells: probiotics and their antigenic metabolites can be phagocytosed by microfold cells forming endosomes that can be released and acquired by dendritic cells, which then transports them to local lymph nodes [72] • Induces the release of antimicrobial defensins from epithelial cells [72] • Modulates innate and adaptive immune responses, and facilitates the development and maturation of the immune system [72] • Regulates host-pathogen interactions by initiating innate immune responses; composed of Toll-like receptors, nuclear factor kappa B (NF-κB), MAPK, and c-Jun NH2-terminal kinase (JNK) pathways [72] • Enhances viability of natural killer cells and macrophages [72] • Stimulates release of secretory IgA [72]
Omega-3 fatty acids	• Upregulates the activation status of macrophages, neutrophils, T-cells, B-cells, dendritic cells, natural killer cells, mast cells, basophils, and eosinophils [73] • Modulates neutrophil function via neutrophil migration, phagocytic capacity, and production of ROS and cytokines [74] • Activates function of T cells by promoting antigen-presenting cells (APC) [73] • Improves function of macrophages by secreting cytokines and chemokines, promoting phagocytosis, and activating macrophages via polarization [73] • Downregulates NF-κB [73] • Anti-inflammatory due to production of different prostaglandins, lipoxins, and peroxisome proliferator-activated receptor gamma (PPARγ) [73] • Affects cell signaling by affecting lipid raft formation and functions [73]

Table 2. *Cont.*

Immunoceutical	Immunomodulatory Properties
Melatonin	• Potent free radical scavenger and antioxidant; detoxifies various ROS and reactive nitrogen species [75] • Stimulates the activities of several antioxidant enzymes and/or upregulates their gene expression [75] • Suppresses activity or downregulates gene expressions of several proinflammatory enzymes (e.g., COX2, inducible nitric oxide synthase (iNOS), eosinophilic peroxidase, and matrix metallopeptidase 2 and 9 (MMP2,9)) [75] • Suppresses NLRP3 inflammasome progression [75] • Inhibition of IκBα phosphorylation suppresses the cytokine storm [75] • Downregulates the overreaction of the innate immune response and promotes adaptive immunity [75] • Inhibits migration of neutrophils to inflammatory sites by blocking ERK phosphorylation [75] • Downregulates mast cell activation, ↓ production of TNF-∝ and IL-6, and inhibits IKK/NF-κB signal transduction pathway in activated mast cells [75] • Balances ratio of T lymphocyte subpopulations and ↑ numbers of B lymphocyte and antibody titer following vaccination [75]

Vit D3 = vitamin D3/cholecalciferol; VDR = vitamin D receptor; CD = cluster of differentiation; TNF-∝ = tumour necrosis factor alpha; IFN = interferon; IFN-γ = interferon gamma; IL = interleukin; MHC = major histocompatibility complex; TGF-β = transforming growth factor beta; RAS = rennin angiotensin system; PSK = polysaccharide K; PSP = polysaccharide-peptides; CV = coriolus versicolor; COX = cyclooxygenase; LOX = lipoxygenase; ERK2 = extracellular regulated kinase 2 MAPK = mitogen-activated protein kinase; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2; Th = T helper cell; APC = antigen presenting cell; ROS = reactive oxygen species; IKKβ = IκB kinase β; NF-κB = nuclear factor kappa B; JNK = c-Jun NH2-terminal kinase; IgA = immunoglobulin A; PPARγ = peroxisome proliferator-activated receptor gamma; MMP = matrix metallopeptidase; iNOS = inducible nitric oxide synthase. ↑ = increased; ↓ = decreased.

2.1. Vitamin D and Immunomodulation

Vitamin D is not only a vitamin, but also a prohormone that acts as an immunomodulator and plays a vital role in maintaining calcium and bone homeostasis. It can be obtained from ultraviolet (UV) B-dependent endogenous production and/or from diet and supplements. Natural dietary sources of vitamin D include fatty fish such as salmon, mackerel, sardines and cod liver oil, and certain types of mushrooms, such as Shiitake, may contain relevant amounts of cholecalciferol or ergocalciferol (two major forms of vitamin D), especially if they are sun-dried [76]. In countries such as the United States and Canada, certain products such as dairy products may also be fortified with vitamin D, usually in the form of cow's milk in the United States, and fluid milk (milk processed for beverage use) and margarine in Canada [77]. Therefore, vitamin D status between individuals will vary greatly depending on the extent of endogenous vitamin D production, which is partly influenced by genetics, latitude of residence, season, concentration of skin pigments and lifestyle (e.g., use of sunscreen and clothing choice), as well as their nutritional habits [78].

Vitamin D exists in two major forms, ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3; 25-hydroxyvitamin D3 (25(OH)D3). Ergocalciferol is derived from the sterol ergosterol (previtamin D2) which is found in plants and fungi. In contrast, vitamin D3 is produced in the skin from 7-dehydrocholesterol when exposed to UVB radiation. Regardless of the form of vitamin D present, it must first be converted to calcitriol ($1,25(OH)_2D$) before it can be biologically active and affect mineral metabolism and modulate the immune system [79]. After consumption, vitamin D is transported in the blood while bound primarily to the vitamin D binding protein (VDR), to the liver where it is converted to calcidiol (25(OH)D3) by the enzymes CYP2R1 and CYP27A1 [78]. Calcidiol is subsequently transported to the kidneys where it is converted into bioactive calcitriol by CYP27B1. Since calcidiol is the main circulating vitamin D metabolite, it is the most reliable parameter for determining vitamin D status in humans [80]. Although CYP27B1 is predominantly found in the kidneys, it has also been found in the placenta and leukocytes including monocytes, macrophages, dendritic cells (DCs) and B and T cells [81–85].

The expression of CYP27B1 by monocytes, macrophages and DCs is critically important since it allows them to convert inactive vitamin D into bioactive vitamin D [78]. Additionally, unlike renal CYP27B1, which is regulated by a negative feedback mechanism, CYP27B1 in monocytes, macrophages and DCs lacks a feedback mechanism, which allows these cells to produce high concentrations of calcitriol locally for immunomodulation (Figure 3) [78].

Vitamin D's role in regulating calcium and phosphorus metabolism, as well as bone health has long been established. However, in recent decades, it has become evident that the functions of this vitamin also extend to the immune system, as demonstrated by the discovery of 1,25-dihydroxyvitamin D3 ($1,25\text{-}(OH)_2D3$) receptors (VDR) in human blood-derived monocytes and active lymphocytes [86]. VDR expression has since been found in almost all cells of the immune system but are expressed at significantly higher concentrations in T-lymphocytes and macrophages, and even higher in developing T cells in the thymus, and established CD8+ cells [87,88]. The effect of vitamin D on different types of leukocytes differs greatly as control of VDR expression is based on the activation status of each type of leukocyte [88]. For example, expression of VDR in monocytes will decrease once they differentiate into macrophages or DCs. In contrast, upon activation, T cells will display a significantly higher concentration of VDR eight hours post-activation, reaching maximum concentration at 48 h [85,89]. Regardless of the different effects that vitamin D has on various leukocytes, a deficiency of vitamin D will result in insufficient and impaired innate and acquired responses, which consequently increases risk of infections [90].

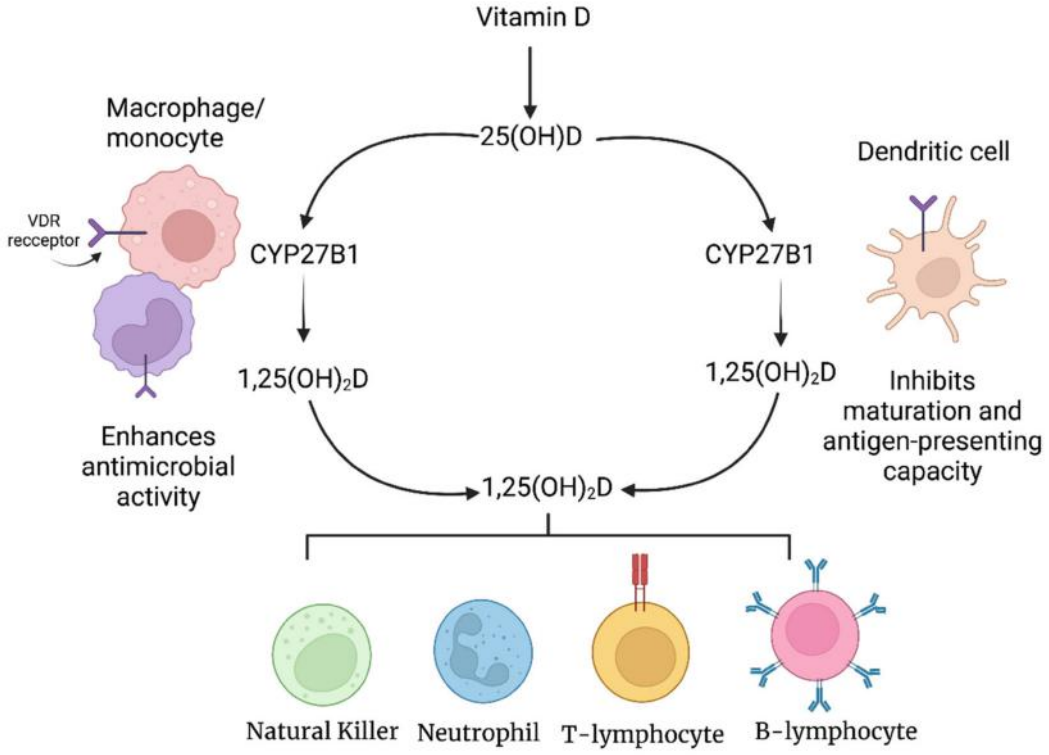

Figure 3. Effects of vitamin D on key cells of the immune system.

2.1.1. Vitamin D and the Innate Immune System

In addition to the expression of VDR in monocytes and macrophages, expression of 1 ∝-hydroxylase, the last enzyme required for the activation of vitamin D, is upregulated in monocytes and macrophages by immunological signaling components, such as signal transducer and activator of transcription-1 (STAT-1∝), interferon-γ (IFN-γ) and Toll-like receptors (TLR) and their ligands (e.g., lipopolysaccharide; LPS) [83,91]. In vitro data also show 1,25-$(OH)_2$D3′s anti-inflammatory activity on macrophages, as is demonstrated by its ability to increase concentrations of IL-10 and decrease the concentrations of inflammatory stimuli such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF), receptor activator of nuclear factor kappa-B ligand (RANKL) and cyclooxygenase (COX)-2 [92]. 1,25-$(OH)_2$D3 inhibits inflammatory cytokines via two pathways. The first involves upregulation of mitogen-activated protein kinase (MAPK)-1 phosphatase by 1,25-$(OH)_2$D3, thus resulting in the subsequent inhibition of LPS-induced activation of p38 [93]. The second pathway involves targeting the thioesterase superfamily member 4 to inhibit COX-2 expression. Additionally, in vitro studies have demonstrated that 1,25-$(OH)_2$D3 also plays a direct role in the antimicrobial activity of monocytes and macrophages by inducing the expression of cathelicidin antimicrobial peptide (CAMP), consequently resulting in the increased expression of hCAP18 and therefore production of the antimicrobial peptide LL-37 [94–96].

1,25-$(OH)_2$D3 affects DCs differently from monocytes and macrophages by attenuating DCs towards a less mature and more tolerogenic phenotype, which is accompanied by unique morphological characteristics [88]. DCs are a unique type of antigen-presenting cell (APC) that not only possesses the ability to initiate and direct innate and adaptive immune responses but are also capable of inducing immunological tolerance; the process by which the immune system does not elicit a response against self-antigens [97]. Tolerogenic DCs possess a reduced capacity to process and present antigens and fully activate T cells [97]. Exposure of differentiating DCs to 1,25-$(OH)_2$D3 in vitro has been shown to interfere with their differentiation and maturation, consequently locking the cells in a semimature state [98]. Tolerogenic mature DCs treated with 1,25-$(OH)_2$D3 are no longer able to activate autoreactive T cells and stimulate the generation of regulatory T cells (Tregs) [99–103].

VDR is also expressed by natural killer (NK) cells and neutrophils [104]. 1,25-$(OH)_2$D3 helps to minimize damage to neutrophils caused by pathogens by downregulating inflammatory cytokine production, while simultaneously increasing their effector function against pathogens by increasing the expression of cathelicidin and ∝ and β-defensins [105,106].

2.1.2. Vitamin D and the Adaptive Immune System

1,25-$(OH)_2$D3 has been shown to affect T-cells directly and indirectly. 1,25-$(OH)_2$D3 indirectly influences T-cells by modulating the stimulatory function of APCs [88]; it does so by downregulating the surface expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules (e.g., CD40, CD80 and CD86) on monocytes and macrophages and DCs, thereby decreasing the potential for antigen presentation [107]. 1,25-$(OH)_2$D3 also specifically affects DCs by inhibiting the production of IL-12 and IL-23, cytokines driving differentiation of helper T cells (Th) into Th1 and Th17 phenotypes, respectively, and stimulates the release of anti-inflammatory interleukin (IL)-10 and macrophage inflammatory protein (MIP)-3∝, which recruits CCR4-expressing Tregs [85]. These indirect effects of 1,25-$(OH)_2$D3 on monocytes, macrophages and DCs can inhibit the proliferation of autoreactive T-cells, induce early and late apoptosis of autoreactive T-cells, and increase the number of Tregs [100,108]. 1,25$(OH)_2$D3 also modulates DC-derived cytokine and chemokine expression by inhibiting the production of pro-inflammatory IL-12 and IL-23, while enhancing the release of anti-inflammatory IL-10 [85,109]. 1,25-$(OH)_2$D3's direct effect on T-cells is variable and dependent upon the activation state of the T-lymphocytes. For example, active T-cells display a higher concentration of VDR, thus allowing 1,25-$(OH)_2$D3 to exert a greater effect on these cell [110,111]. 1,25-$(OH)_2$D3 has been shown to inhibit the production of Th1-associated cytokines (e.g., IL-2, IFN-γ), Th17 cytokines (e.g., IL-17, IL21) and Th9 cytokines (e.g., IL-9) [109,112,113].

The expression of VDR on B cells also suggests that vitamin D has an influence on their function. In vitro data show that 1,25-$(OH)_2$D3 induces apoptosis of activated B-cells and hinders their differentiation to plasma cells and memory B-cells [114]. 1,25-$(OH)_2$D3 also upregulates production of IL-10 by B-cells [115]. Finally, 1,25-$(OH)_2$D3 downregulates the expression of CD86, and upregulates the expression of CD74, which indirectly reduces the activation of T-cells by dampening the APC function of B cells [92,116].

2.1.3. Vitamin D and Autoimmune Disease

Studies have demonstrated a positive correlation between vitamin D status and risk of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus (SLE) [117]. In a study conducted by Deluca and Cantorna [87], 1,25-$(OH)_2$D3 was found to either prevent or suppress autoimmune encephalomyelitis, SLE, type I diabetes and inflammatory bowel disease, which are all autoimmune diseases characterized by hyperactive T cell-mediated immunity. A possible mechanism by which vitamin D prevents or suppresses autoimmune disorders involving hyperactive T cells is by stimulating the production of transforming growth factor (TGF)-β and IL-4, which suppress T cell- mediated inflammatory responses [87].

Vitamin D is unique among immunoceuticals in that it supports both early immunological activation events and innate host defence mechanisms such as production of defensins, while subsequently also helping to restore immune system homeostasis following activation of adaptive immune responses, by promoting recruitment of Tregs and production of immunosuppressive cytokines.

2.1.4. Vitamin D Dosing

It is important to note that while many countries may have guidelines for recommended daily dietary vitamin D intake, these are designed for optimal bone health rather than for optimal functioning of the immune system. The immune system, however, is generally the most metabolically active in the body, especially when responding to infections or other diseases. Indeed, activated T and B cells can proliferate at rates equivalent to the

most rapidly dividing cancer cells. Consequently, requirements for vitamin D could be as high as 60,000 IU/day over a short-term period to ensure optimal immune responses [118].

The current dietary reference intakes (DRI) for vitamin D set out by Health Canada and the US National Institutes of Health (NIH) are solely based on maintaining skeletal health and preventing rickets. Furthermore, Health Canada has confusing guidelines, with one version having a recommended dietary allowance for vitamin D of 600 international units (IU) per day for people 9–70 years of age [119]. The other published guideline recommends 400 IU per day for people 2+ years of age [120].

According to Health Canada's website on vitamin D and calcium, due to the inconsistent evidence that exists to date and the absence of a cause-and-effect relationship regarding various health outcomes potentially related to calcium and vitamin D, such as cancers, cardiovascular disease, diabetes, and immunity, these health outcomes were not used in determining the updated vitamin D and calcium recommended intake guidelines by the U.S. Institute of Medicine (IOM) [119]. However, because evidence surrounding calcium and vitamin D's role in bone health was deemed to be convincing by the IOM expert committee, the determination of calcium and vitamin D requirements were based on bone health [119]. Furthermore, the listed tolerable upper intake level (UL) per day by Health Canada and the NIH vastly underestimates the concentration of vitamin D that is considered safe for most individuals. For example, the UL for children and adults from 9–70 years of age set out by Health Canada is 4000 IU. Canadians are also advised that intakes of vitamin D above the new recommended dietary allowance does not offer any additional health benefits, and any intake of vitamin D above the new UL may result in possible adverse effects. Possible adverse effects in this case refer to vitamin D toxicity, which is characterized by an excess amount of calcium being deposited in the body, leading to the calcification of the kidney and other soft tissues including the heart, lungs, and blood vessels. In contrast, numerous studies regarding the use of vitamin D for treating influenza, COVID-19, and pneumonia have consistently shown that vitamin D doses of up to 10,000 IU/day are considered safe for the vast majority of patients [121]. While the IOM also recognizes that no adverse effects have been reported from studies involving the supplementation of less than 10,000 IU/day of vitamin D, the UL is still set at 4000 IU/day due to the presence of U-shaped 25(OH)D concentration-health outcome relationships found in observational studies. However, later investigations have since determined that observational studies reporting J-or U-shaped relationships, did not measure baseline serum 25(OH)D concentrations of participants and thus may have enrolled participants that were already taking vitamin D supplements prior to the study [122]. These U- or J-shaped associations between 25(H)D concentration and health outcomes indicate that the higher the baseline vitamin D status, the greater the risk of adverse outcomes if intake of additional vitamin D for treatment purposes is not adjusted [122].

However, it should be noted that the threshold of toxicity varies from individual to individual as it is highly dependent on lifestyle, diet, skin color, skin type, age, etc. An obese middle-aged individual, for example, with little to no exposure to sunlight, and who consumes a low vitamin D diet will require a much higher dosage of vitamin D to reach toxic concentrations in comparison to a healthy individual in their early 20s with frequent exposure to sunlight and who consumes a balanced diet. In addition to concentration, intake duration of vitamin D must also be taken into consideration when discussing vitamin D toxicity. A single high dose of vitamin D (e.g., 200,000 to 300,000 IU) will not cause adverse effects, as one would need to take daily doses of 25,000 IU of vitamin D for several months or 1 million IU of vitamin D for several days for toxicity to occur [123].

An alternative and more accurate method of determining vitamin D's safe upper limit is by assessing serum 25(OH)D concentrations (Table 3) [121,124–128].

Table 3. Vitamin D status categorized by serum 25(OH)D concentrations.

Vitamin D Status	Serum 25(OH)D Concentrations (nmol/L)	Serum 25(OH)D Concentrations (ng/mL)
Severe deficiency	<25	<10
Moderate deficiency	25–<50	10–<20
Insufficiency	50–<75	20–<30
Sufficiency	75–<100	30–<40
Optimal concentration	100–<150	40–<60
Increased concentration	150–<250	60–<100
Overdose	≥250	≥100
Intoxication	≥375	≥150

According to the U.S. IOM 2011 report, which was jointly commissioned and funded by the U.S. and Canadian governments, a serum 25(OH)D concentration of 20 ng/mL (50 nmol/L) or higher is considered adequate for optimal bone health [121]. However, there is increasing evidence that suggests serum 25(OH)D levels of 75 nmol/L and above are sufficient to ensure normal skeletal and muscular structure and function [125,126,129,130]. Furthermore, increasing evidence also suggests that a minimum serum concentration of 100 nmol/L of 25(OH)D is needed to reduce the risk of certain cancers (e.g., colorectal), cardiovascular disease, infectious diseases, pathological pregnancies (e.g., preeclampsia, gestational diabetes, preterm birth), systemic connective tissue diseases, diabetes and COVID-19 [121,123,131–138]. Thus, a serum concentration of at least 100 nmol/L of 25(OH)D appears to be optimal for supporting all systems of the body and not only the skeletal system.

2.2. Medicinal Mushrooms and Their Immunomodulatory Properties

Certain mushroom species have long been identified to display profound health promoting benefits. The practice of using mushrooms in Chinese traditional medicine can be dated back to as early as 200–300 AD [139]. Mushroom-producing technologies have greatly advanced to meet the global mushroom market, which was valued at 50.3 billion USD in 2021, and it is expected to expand at a compound annual growth rate of 9.7% from 2022 to 2030 [140]. Examples of medicinal mushrooms include *Lentinula edodes* (Shiitake), *Grifola frondosa* (Maitaki), *Flammulina velutipes* (Enoki), *Pleurotus* (spp) (Osyter), *Ganoderma lucidum* (Reishi), *Trametes versicolor* [139]. *Ganoderma lucidum* and *Trametes versicolor* are not palatable due to their bitter taste and coarse texture, however, they are traditionally used medicinally as hot water extracts [139].

Lentinan is a β-glucan cell wall component extracted from the mushroom *Lentinula edodes* [139,141]. As β-glucans are not found in animals, they can stimulate the immune system by activating various leukocytes including macrophages, DCs, neutrophils, NK cells and lymphocytes [141]. There are several leukocyte surface receptors (e.g., Dectin-1, TLRs, complement receptor type 3, scavenger receptors and lactosyceramide (LacCer)) that recognize β-glucans as non-self molecules, consequently inducing the innate and adaptive immune response [142,143]. Dectin-1 is commonly expressed on neutrophils, DCs, and certain T-cells. The binding of β-glucans to these pattern recognition receptors activates several signaling pathways that promote innate immune responses, including the induction of inflammatory cytokines, activation of phagocytosis and production of reactive oxygen species [141]. The binding of lentinan to TLRs can lead to the production of various cytokines such as IL-2 and IL-12 [141]. The receptor LacCer can be found on neutrophils and endothelial cells, and it is purported that the interaction of lentinan with LacCer will induce production of MIP-2, activation of nuclear factor-κB, and neutrophil oxidative burst [144–146].

Polysaccharide krestin (PSK) and polysaccharide peptide (PSP) are protein-bound polysaccharides that are derived from different strains of the mushroom *Trametes versicolor*, also known as Yun Zhi, or turkey tail. PSP and PSK are chemically similar and exert

similar physiological effects. The difference between the two lies mainly in the presence of fucose in PSK, and rhamnose and arabinose in PSP [139]. Both PSP and PSK are potent immunostimulators and can increase white blood cell counts, production of IFN-γ and IL-2, and delayed type hypersensitivity reactions [147] suggesting they possess adjuvant-like properties.

There is substantial evidence to suggest that PSP predominantly induces pro-inflammatory cytokines [62]. Both *in vivo* and *in vitro* studies have demonstrated PSP's potent effect on expression of TNF-∝. For example, in a study conducted by Chan and Yeung [148], the in vitro treatment of mouse peritoneal macrophages with PSP increased the release of TNF-∝ to concentrations comparable after induction by LPS. Cytokines associated with TNF-∝, can also be induced by PSP, as is evident by its induction of IL-12, a Th-1-related cytokine that enhances NK and CD8+ T cell cytotoxic activities and their expression of TNF-∝ [62]. Additionally, the expression of other pleiotropic cytokines, such as TGF-β have been shown to be affected by PSP [62]. Findings also suggests that in addition to PSP's ability to directly affect cytokine release by leukocytes, PSP also increases the sensitivity of leukocytes to other stimuli, sometimes acting synergistically [62]. Moreover, findings from a study conducted by Li [149] demonstrated that when PSP is added at different concentrations to human blood and mouse splenocytes, it promoted T cell proliferation. Augmentation of Th cell activation, as well as an increase in the ratio of Th cell (CD4+)/T suppressor (CD8+) was also observed following the administration of PSP in the same study.

The administration of PSK under normal physiological conditions has been shown to have no substantial effect on host immune responses [150,151]. However, PSK can help to restore the immune system back to homeostasis following immunological depression caused by tumor burden or chemotherapy [151–153]. For example, Harada et al. [154] demonstrated that the oral administration of PSK improved the impaired anti-tumor CD4+ T-cell response in the gut-associated lymphoid tissue of specific pathogen free mice. Kato et al. [155] and Liu et al. [156] also reported that PSK can induce the expression of certain cytokine genes in vivo and in vitro, including TNF-∝, IL-1, IL-8, and IL-6; these cytokines mediate multiple biological effects in part via their direct stimulation of cytotoxic T cells against tumors, enhancement of antibody production by plasma cells and induction of IL-2 receptor expression on T lymphocytes.

In addition to their immunomodulatory properties, the in vitro anticancer activity of both PSP and PSK on human cancer cell lines and in human clinical trials is quite extensive [139]. However, a complete description of their anticancer activities is outside the scope of this review.

2.3. Immunomodulatory Properties of Quercetin and Kaempferol

Quercetin and kaempferol are flavonols, a subgroup of flavonoids, a family of polyphenolic compounds that are found abundantly in a variety of fruits and vegetables, including onions, kale, lettuce, tomatoes, apples, grapes, and berries [157], as well as some types of tea [158] and wine [159]. Of note, onions are qualitatively and quantitatively the most important dietary source of quercetin [160], whereas green leafy vegetables such as spinach, kale, dill, chives, and tarragon are the richest plant sources of kaempferol [160].

Quercetin and kaempferol are typically conjugated to simple sugars, such as glucose, xylose, rhamnose, arabinose or galactose or disaccharides (e.g., rutinose) in plants [161,162]. The broad spectrum of health-promoting effects associated with flavonoids make them an essential component of many nutraceutical, pharmaceutical, medicinal and cosmetic applications [157]. Flavonoids are known to possess antioxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic, and immunomodulatory properties, as well as the ability to modulate key cellular enzyme functions, including the inhibition of the enzymes xanthine oxidase, COX, lipoxygenase (LOX) and phosphoinositide 3-kinase [163–165]. For this review, the anti-inflammatory properties of quercetin and kaempferol will be considered as immunomodulatory. The recognition of viruses, bacteria, parasites, antigenic substances and/or chemicals via various cell receptors will activate many inflammatory

pathways, resulting in the production of cytokines and activation of leukocyte subsets, such as macrophages and lymphocytes, to eliminate foreign bodies. If the early phase of inflammation fails to eliminate the foreign bodies, an increased production of cytokines, chemokines and inflammatory enzymes may drive inflammation into a chronic phase. The inflammatory pathway is regulated by many receptor-mediated pathways, including TLRs, MAPK pathways and the nuclear factor kappa-light chain enhancer of activated B cells, which a transcription factor that regulates more than 50 genes involved in inflammation. The deregulation of any of these pathways results in the onset and progression of various inflammatory disorders. Quercetin and kaempferol, as well as other flavonoids and polyphenols with anti-inflammatory activities can interact with many molecules involved in the inflammatory pathway to decrease the activity of cytokines, chemokines, and inflammatory enzymes.

Quercetin and kaempferol are known to modulate both innate and adaptive immune responses, exerting stimulatory and inhibitory effects on different types of leukocyte sub-populations and pathways, particularly the inflammatory pathway [166]. The structure of quercetin and kaempferol, and flavonoids in general, is what imparts their anti-inflammatory properties [167]. Quercetin has been shown to reduce inflammation by inhibiting c-Jun N terminal kinase and extracellular signal-regulated kinase, consequently inhibiting MAPK and the transcription factor activator protein -1 and nuclear factor-kB activity [167]. Quercetin is also capable of enhancing the production of IL-10, an anti-inflammatory compound, by inhibiting IL-1β and TNF-∝ [168]. Additionally, quercetin influences the expression of adhesion molecules (e.g., vascular cell adhesion protein 1) and the release of metalloproteinases, thereby reducing inflammation-mediated tissue damage [70]. The anti-inflammatory activities of quercetin are also due its inhibition of inflammation-promoting enzymes, such as COX and LOX, as well as down-regulating nitric oxide (NO) production and/or inducible nitric oxide synthase (iNOS) enzyme expression and activity [169,170], as well as inflammatory mediators [160]. Furthermore, quercetin is known to inhibit the maturation of DCs (derived from murine bone marrow) and their expression of MHC molecules, thereby reducing antigen uptake and the secretion of pro-inflammatory cytokines (IL-1, IL-2, IL-6 and IL-12) [171–174].Taken together, quercetin modulates immunity and inflammation by mainly acting on DCs and targeting the many intracellular signaling kinases and phosphatases, enzymes and membrane proteins that are often crucial for a specific cellular function [175].

In addition to quercetin's anti-inflammatory properties, there is also a large body of evidence in the literature to support quercetin's anti-allergic properties both in vitro and in vivo. Quercetin's anti-allergic properties, just like its anti-inflammatory properties are directly correlated with its modulation of the immune system. Quercetin is purported to inhibit the production and release of histamine and other allergic and inflammatory substances by stabilizing the cell membranes of mast cells [176,177]. Mast cells play a vital role in the pathogenesis of allergic responses and autoimmune disorders, and they affect the release of many cytokines involved in inflammatory reactions, such as IL-8 and TNF-∝ [178,179]. As such, quercetin is well suited for the treatment of mast cell-derived allergic inflammatory diseases, including asthma, sinusitis, and rheumatoid arthritis [163]. Quercetin is also a known inhibitor of allergic (IgE-mediated) mediator release from mast cells and basophils, and an inhibitor of human mast cell activation [160,180].

Kaempferol, in contrast, exerts its anti-inflammatory activities by inhibiting the nuclear factor-kB binding activity of DNA and myeloid differentiation factor 88, suppressing the release of IL-6, IL-1β, IL-18 and TNF-∝, increasing mRNA and protein expression of Nrf2-regulated genes and inhibiting TLR4 [181–184]. Like quercetin, kaempferol's anti-inflammatory properties are also in part due to its inhibition of COX and LOX, pro-inflammatory enzymes, as well as the inhibition of NO production and/or iNOS enzyme expression and activity [168]. Kaempferol also has an immunosuppressive effect on DCs by attenuating their activation [185]. The disruption of nuclear factor- kB and the MAPK pathways, as well as the suppression of calcineurin by kaempferol is one possible mechanism by

which kaempferol inhibits DC function [186–190]. Another possible mechanism involves the peroxisome proliferator-activated receptor (PPAR) γ, a transcription factor involved in the anti-inflammatory response, as kaempferol is known to be a potent stimulator of PPARγ activity [190].

2.4. Immunomodulatory Properties of Curcumin and Resveratrol

Curcumin is an extract of the rhizome of turmeric (*Curcuma longa* Linn) [191], and its antiangiogenic, antiproliferative, antitumorigenic, antioxidant, and anti-inflammatory properties have been investigated in both in vitro and in vivo studies [192]. Resveratrol [193] is a well-known biologically active compound synthesized by plants and initially isolated from white hellebore (*Veratrum grandiflorum* O. Loes) roots used in traditional Chinese and Japanese Medicine as an anti-inflammatory and anti-platelet agent. Curcumin and resveratrol protect cells from oxidative stress [194], which could have a role in preventing inflammatory disorders [195], such as cancer, inflammation, and diabetes. Curcumin and resveratrol have been widely reported to have anticancer properties [192,196], and evidence suggests the significance of these phytochemicals as preventive and therapeutic agents that can effectively target CRC development and progression [197]. However, it has been documented that these polyphenols exhibited immunosuppression activities by the down-regulation of costimulatory molecule expression, CD28, and CD80; up-regulating CTLA-4 on macrophages; and the augmentation of the IL-10 generation. Both compounds suppress the activity of T and B cells, as evidenced by significant inhibition in proliferation, antibody production, and lymphokine secretion [198]. Further studies are required to dissect these phytochemicals' chemopreventive effects and their anticancer mechanisms.

Cellular inflammatory mediator secretion is under the regulation of different signaling pathways, including the IκB kinase β (IKKβ) and NF-κB pathways [199]. It has been shown that curcumin and resveratrol suppress NF-κB-regulated gene products involved in osteoarthritis, and IL-1β-induced NF-κB activation was shown to be suppressed directly by cocktails of curcumin and resveratrol [200]. We have also reported that treating human macrophages with curcumin inhibits IL-8 production by 85% (from ~52.5 ± 7 ng/mL to ~4.2 ± 0.3 ng/mL) via the suppression of NF-κB upon cigarette smoke exposure [201]. However, studies show that supplementation with curcumin and resveratrol has no impact on the postprandial inflammation response to a high-fat meal in abdominally obese older adults [199], demonstrating that the whole-blood *NFKB1* gene expression and C-reactive protein, as well as the generation of inflammatory cytokines IL-6 and IL-8, stayed unchanged. Conversely, the oral administration of resveratrol and curcumin ameliorates acute small intestinal inflammation by down-regulating Th1-type immune responses and prevents bacterial translocation by maintaining gut barrier function [202]. Other studies [203] have shown that curcumin and resveratrol could regulate weaned piglet gut microbiota, down-regulate the TLR4 signaling pathway, alleviate intestinal inflammation, and ultimately increase intestinal immune function. These findings indicate that the gut microbial metabolites of the polyphenol supplementation, which contribute to the clinical effects, need to be investigated.

Considering all the research conducted so far, there is no doubt that curcumin and resveratrol are preventive/therapeutic agents that can be used in the management and treatment of cancer and inflammatory diseases. However, further investigation should clarify how these classes of dietary micronutrients should be administered to maximize their anti-carcinogenic, anti-angiogenic, pro-apoptotic, or anti-oxidative effects and enhance their beneficial effects in the prevention or control of several diseases.

3. Conclusions

There is a significant and growing need for innovative and alternative treatment options as well as novel healthful products to prevent and/or treat the increasing number of infectious diseases, cancers, autoimmune disorders, inflammatory conditions, and allergies affecting both human and animal populations in a more natural manner. As the nutraceu-

tical market expands, it is becoming increasingly clear that more studies are needed to potentially establish new definitions of sufficiency and deficiency based on immunological health parameters, as well as for the defining and testing of immunocompetence. Currently, all recommended nutrient intake guidelines are based on preventing deficiency. With regard to vitamin D, for example, the current recommended intake guidelines set out by Health Canada and the FDA are solely based on maintaining skeletal health and preventing rickets and have nothing to do with optimizing the function of the most metabolically active system in the body, the immune system. Indeed, the immune system requires a much higher vitamin D intake (e.g., as much as 4000–10,000 IU versus 400–600 IU). Furthermore, the concentration of vitamin D required for an optimal immunological function for one individual differs from another due to lifestyle (e.g., use of sunscreen and clothing choice), level of skin pigmentation, exposure to the sun, nutritional habits, and genetics, as well as the season and geographical latitude of residence. As such, there is a need for high-quality studies to assess the roles of vitamin D in optimizing immunological functions, especially in clinically relevant contexts and with precise measurements of serum concentrations. Defining and standardizing testing for immunocompetency would allow for a more focused, personalized approach to supporting the immune system and ensuring its optimal functioning to minimize the acquisition of diseases.

Additionally, as the list of potential immunoceuticals expands, additional studies are required to determine their mechanisms of action on the immune system, the concentrations at which they exert their immunomodulatory properties, optimal methods of delivery, how well in vitro studies translate into in vivo results, and whether they can serve as standalone treatment options or act as adjunctive therapies for certain pathological conditions and disorders. Although standardized methods, such as the High Immune Response technology, are available for livestock, more studies are required to determine if certain immunoceuticals can be used in various animal species to help treat, prevent, or augment the treatment of common and highly infectious diseases afflicting the livestock industry, including respiratory diseases in swine, dairy, beef, and poultry, as well as mastitis, weaning disorders, and parasitic infections, to name a few. The immunoceuticals industry is full of potential to provide natural, economical, and efficacious alternatives to traditional pharmaceuticals and fill therapeutic gaps that currently exist in the drug industry. In short, they have the potential to revolutionize how pathological conditions are treated or prevented through the modulation of the immune system.

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This is Exhibit X referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

antioxidants

MDPI

Review

N-Acetylcysteine and Its Immunomodulatory Properties in Humans and Domesticated Animals

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Abstract: N-acetylcysteine (NAC), an acetylated derivative of the amino acid L-cysteine, has been widely used as a mucolytic agent and antidote for acetaminophen overdose since the 1960s and the 1980s, respectively. NAC possesses antioxidant, cytoprotective, anti-inflammatory, antimicrobial, and mucolytic properties, making it a promising therapeutic agent for a wide range of diseases in both humans and domesticated animals. Oxidative stress and inflammation play a major role in the onset and progression of all these diseases. NAC's primary role is to replenish glutathione (GSH) stores, the master antioxidant in all tissues; however, it can also reduce levels of pro-inflammatory tumor necrosis factor-alpha (TNF-∝) and interleukins (IL-6 and IL-1β), inhibit the formation of microbial biofilms and destroy biofilms, and break down disulfide bonds between mucin molecules. Many experimental studies have been conducted on the use of NAC to address a wide range of pathological conditions; however, its effectiveness in clinical trials remains limited and studies often have conflicting results. The purpose of this review is to provide a concise overview of promising NAC usages for the treatment of different human and domestic animal disorders.

Keywords: N-acetylcysteine; antioxidant; cytoprotective; immunomodulatory agent; oxidative stress; glutathione

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1. Introduction

N-acetylcysteine (NAC) comes from the amino acid L-cysteine and has been a Food and Drug Administration (FDA) approved drug since 1963. It is recognized as the standard treatment for acetaminophen overdose and has been used as a mucolytic drug since the 1960s [1]. NAC has also been used as a supplement for decades and, as such, can be found as an over-the-counter nutritional supplement in countries such as the United States, Canada, and Australia [1]. Therefore, NAC falls into a grey zone with other compounds such as cannabidiol (CBD), but as of August 2022, the FDA has backed away from its hard stance on classifying NAC as a drug and is allowing its sale as a dietary supplement [2]. NAC's antioxidant and anti-inflammatory properties make it a promising therapeutic agent for conditions in which oxidative stress is involved [1]. Examples of these disorders include diabetes, obesity, cancer, neurological disorders, hypertension, pulmonary, inflammatory bowel, cardiovascular, autoimmune, and infectious diseases in humans, as well as domesticated animal weaning disorders, respiratory disease, diarrhea, endometritis, and mastitis [3–10].

While there has been an increasing interest in the use of NAC for a wide range of pathological conditions over the past few decades, various human clinical studies have

reported conflicting results, and many other studies were performed in vitro. Therefore, more clinical studies are required to address these conflicting results and to support the purported therapeutic roles of NAC in treating different pathological conditions.

Various animal studies involving the use of NAC have also been performed, especially with its use in treating intestinal inflammation and related disorders that can be caused by anti-nutritional factors (e.g., β-conglycinin, a vicilin storage protein of soybeans), the process of weaning, and consumption of mycotoxins [10–12]. Many studies have shown the protective effects of NAC for neonatal animals immune challenged with bacterial lipopolysaccharide (LPS) endotoxin, particularly studies involving piglets [13,14]. NAC administration has consistently been shown to increase daily body weight gain and alleviate LPS-mediated growth depression. Furthermore, the porcine model of ulcerative colitis (UC) can be used to support NAC usage for humans. For example, in a study conducted by Wang et al. (2013), the piglet model of UC demonstrated that administration of NAC was able to reduce colon histopathology score and ameliorate UC histological abnormalities [15].

Despite the numerous NAC studies and its increasing popularity, the mechanisms of action (MOA) by which NAC exerts its antioxidant and cytoprotective properties remain unclear [1,16]. It is often assumed that the effects conferred by NAC are due to it acting as a scavenger of reactive oxygen species (ROS), a precursor for glutathione (GSH) biosynthesis, and a disulfide reductant [16,17]. However, these three major narratives can only explain the effects of NAC under specific circumstances [16,17]. Recently, an alternative MOA was proposed that may explain the effects attributed to NAC: the conversion of NAC into hydrogen sulfide and sulfane sulfur species, which are known to possess antioxidant and cytoprotectant properties [16,17]. Thus, the purpose of this review is to provide an overview of the therapeutic uses of NAC in both humans and domesticated species, with a particular focus on weaning disorders, as well as an overview of its MOA.

NAC Formulations

The most common and well-known formulation of NAC in the United States is Mucomyst™, which is commonly administered orally for the treatment of acetaminophen toxicity [18]. PharmaNAC® (BioAdvantex Pharma Inc., Mississauga, ON, Canada) is another common oral formulation and is the only effervescent preparation of its kind available in North America [19]. Due to its disagreeable flavor, NAC is often mixed with fruit juice or a soft drink prior to consumption. In Europe, NAC is available in pills, capsules, and a variety of effervescent "fizzy tab" formulations [18].

2. Safety Profile of NAC

The safety of NAC has been well-established through numerous pharmacological studies [17]. Toxicity is rare and dependent on the route of NAC administration and dosage [1]. NAC can be administered orally, intravenously, or intranasally [1]. When orally administered, NAC undergoes rapid intestinal absorption and is subsequently metabolized by the liver [1]. The cysteine released during NAC metabolism is utilized for glutathione (GSH) synthesis, which is vital for immune function and tissue repair [1]. NAC's bioavailability following oral administration is less than 10%. Therefore, only a small portion of intact NAC reaches the plasma and tissue [1]. It has been suggested that the low bioavailability of oral NAC may be due to first-pass metabolism in the small intestine rather than incomplete absorption [20]. NAC can be found intact, reduced, or in various oxidized forms in the plasma following oral intake [1,20], and individual variation in these NAC metabolites occurs, which is likely due to natural variations in human metabolism and the intestinal microbiota. Comparatively, intravenous or nasal administration allows for rapid delivery of high concentrations of NAC to the circulation as it bypasses the first-pass intestinal and hepatic metabolic pathway [1]. Considering that NAC bioavailability is influenced by first-pass metabolism, it will be important to address potential gender and age effects, which are known to affect the first-pass metabolism [21,22]. Therefore, an optimal way to maintain a proper therapeutic serum level of NAC after intake should

include monitoring NAC metabolite concentrations in the blood to maximize both the efficacy of treatment and patient safety.

3. Transportation of NAC

NAC is transported into cells through a complex process involving active transport mechanisms. Within cells, NAC is primarily absorbed and utilized in the cytoplasm, where it is converted into cysteine. NAC is observed to have a slower rate of cellular absorption compared to cysteine [23]. This delayed uptake is attributed to the hindrance caused by the N-acetyl group, which affects both passive and active transportation across the cell's plasma membrane. Passive transport of NAC is particularly disadvantaged due to its negative charge at physiological pH, unlike Cys, which is a zwitterion with no net charge, resulting in reduced membrane permeability [24]. Remarkably, modifications neutralizing the charge on NAC's carboxyl group, such as amidation or esterification, have been shown to significantly enhance its cellular uptake, as previously reported [25,26]. Despite these findings, there is currently no substantial evidence suggesting the existence of active membrane transport systems specific to NAC. ASCT1, the canonical importer of reduced Cys, does not transport negatively charged amino acids, including NAC [27]. While there have been reports implicating anion exchanger 1 (AE1) as a potential facilitator of NAC uptake into erythrocytes, this remains an area requiring further investigation, as pointed out by Raftos et al. (2007) [23]. Once absorbed, NAC must undergo deacetylation to yield cysteine. Aminoacylase 1 is believed to be the primary enzyme responsible for deacetylating NAC in this process.

3.1. Adverse Reactions

Adverse effects following administration of NAC range from mild to severe and are dependent on the formulation, concentration, and route of administration [1,28] (Table 1). Intravenous NAC and oral NAC are commonly associated with minimal side-effects [1], such as symptoms of nausea, vomiting, pruritus, and erythema [28,29]. The frequency of NAC side-effects following intravenous administration is significantly higher compared to oral NAC [18]. Inhaled NAC can result in adverse reactions such as bacterial pneumonia, cough, sore throat, and drug-induced pneumonitis, but coughing is the most prevalent [1,30]. Anaphylactic reactions resulting from NAC are rare and mild or moderate depending on the concentration [31]; most are typically attributed to intravenous administration due to the transient but significant increase in NAC plasma levels [18]. Existing data suggests that there is a direct relationship between anaphylactoid reactions and serum NAC concentration [31]. Symptoms of anaphylactoid reactions include flushing, pruritus, angioedema, bronchospasm, and hypotension [32]. These anaphylactoid reactions rapidly subside following the discontinuation of NAC administration or lowering the rate of intravenous administration [18].

3.2. NAC Dosing and Pharmacokinetics

NAC is commonly taken orally at doses of 600–1200 mg daily to treat specific conditions, or even as a dietary supplement. Administration of oral NAC at doses as high as 8000 mg/day is well-tolerated with no clinically significant adverse reactions [33]. NAC has a half-life of 5.58 h after intravenous administration [20]. Its half-life following oral administration is 6.25 h, reaching a maximum plasma concentration (Cmax) approximately 1 to 2 h post administration [20].

4. Therapeutic Uses of NAC in Animals

While studies have been conducted on the therapeutic uses of NAC in livestock and companion animals, in addition to its use as an antidote for acetaminophen poisoning in dogs and cats, limited pharmacokinetic data on NAC exists in the literature for other species. Among the limited pharmacokinetic data available, only two studies discussed the pharmacokinetics of NAC in chickens and healthy cats. In the study conducted by Buur et al.

(2013), the half-life of NAC following IV and oral administration (100 mg/kg) in healthy cats was found to be 0.78 ± 0.16 h and 1.34 ± 0.24 h, respectively, and the bioavailability of NAC following oral administration was 19.3 ± 4.4% [34]. The pharmacokinetics of NAC found in the six cats used in this study differs from the values reported for humans; thus extrapolating dosages from human medicine may lead to underdosing cats with acute disease. This study has limitations, however, given its small population size and the assumption that the pharmacokinetics of NAC in diseased cats are similar to that of healthy cats. In the study conducted by Petkova and Milanova (2021), NAC was found to reach a maximum plasma concentration of 2.26 ± 0.91 g/mL 2.47 ± 0.45 h following oral administration of 100 mg/kg in healthy broilers [35]. NAC's half-life was found to be 1.04 ± 0.53 h. The authors also did not find any significant difference between NAC's pharmacokinetics in healthy broilers versus Mycoplasma gallisepticum-infected broilers.

5. Therapeutic Uses of NAC in Humans

NAC is one of the most used antioxidants in clinical practice to date [16,17,36]. There has been a growing interest over the past few decades to use NAC as a potential treatment for a wide range of diseases and disorders in which oxidative stress is suspected to play a role [17,37], including respiratory, cardiovascular, neurodegenerative, liver, kidney, gastrointestinal, and infectious diseases [1].

Table 1. N-acetylcysteine adverse events.

Study	Study Type	Study Size	NAC Treatment	Type and Incidence of Adverse Events following NAC Treatment		
					Moderate	Severe
[38]	Double-blinded, placebo-controlled, crossover design	17 healthy individuals (age 30 ± 2 years)	9 mg/kg NAC capsule	Upset stomach (2), nausea (1), stomach/intestinal gas (1), cough (1)		
			18 mg/kg NAC capsule	Upset stomach (1), nausea (1), stomach/intestinal gas (2), sleepiness (2), metallic taste (1)		
			35 mg/kg oral NAC solution	Upset stomach (2), stomach/intestinal gas (2), sleepiness (1), metallic taste (1)		
			70 mg/kg oral NAC solution	Upset stomach (2), stomach/intestinal gas (1), sleepiness (3), metallic taste (3), light-headedness (1), cough (1)	Upset stomach (1),	Stomach/intestinal gas (1)
			140 mg/kg oral NAC solution	Upset stomach (5), nausea (3), stomach/intestinal gas (2), sleepiness (2), metallic taste (4), light-headedness (1)	Upset stomach (1), stomach/intestinal gas (4)	
[39]	Randomized, placebo-controlled study	28 individuals (≤75 years)	20 mg/min intravenous NAC for first hour, then 10 mg/min for next 23 h; total dose of 15 g over 24 h	Haemorrhage (3), headache (4)		Transient episode of extreme sinus bradycardia (1)
[40]	Randomized, placebo-controlled study	4 healthy individuals (age 35 ± 3 years)	150 mg/kg intravenous	Transient skin flushing (2), pruritus (2), nausea (2)		
[41]	Double-blind, randomized trial	65 chronic bronchitis patients	4 puffs of NAC (1 mg/puff) two times daily	Coughing (4), Dyspnoea (7)		

5.1. Liver Diseases

Acute and chronic liver diseases are highly prevalent worldwide, accounting for approximately 2 million deaths per year [42]. Oxidative stress plays a crucial role in the initiation and progression of liver diseases due to its participation and stimulation in the liver's fibrogenic response [1]. Numerous clinical and experimental studies have been conducted on the use and efficacy of NAC in modulating inflammation and oxidative stress caused or propagated by liver diseases, including acetaminophen (paracetamol) poisoning, acute liver failure, and non-alcoholic fatty liver (NAFLD) and alcoholic liver diseases (see Table 2).

5.1.1. Acute Acetaminophen Overdose

Acetaminophen is a commonly used over-the-counter analgesic and antipyretic medication as it is relatively safe and effective in treating mild-to-moderate pain and fever at appropriate doses [28,43]. However, when taken by adults at doses of 10–15 g (single or repeated) over a 24 h period, which is 3–5 times the manufacturer's recommended dose for over-the-counter use, acetaminophen has direct hepatotoxic potential and can cause acute liver injury and death from acute liver failure [28,43]. Following ingestion, acetaminophen is rapidly absorbed and transported to the liver to undergo first-pass metabolism [28]. N-acetyl-p-benzoquinonimine (NAPQI), a cytochrome P450-derived metabolic by-product of acetaminophen first-pass metabolism is cytotoxic and genotoxic [28]. Normally, when acetaminophen is taken at low doses, GSH can detoxify NAPQI by conjugating it, which facilitates its elimination from the body [28]. However, acetaminophen toxicity leads to the formation and accumulation of excessive amounts of NAPQI, the depletion of GSH stores, oxidative stress, and mitochondrial dysfunction resulting in the depletion of adenosine triphosphate (ATP) stores [44]. Evidence suggests that NAPQI is capable of binding to several cellular proteins, particularly mitochondrial proteins [44]. This binding to mitochondrial proteins in the context of GSH depletion is of great significance as it results in the depletion of endogenous antioxidant functions and alters the ∝-subunit of the mitochondrial ATP-synthase, thereby hindering the production of ATP [44]. NAC functions to replenish hepatic GSH stores and provide a larger supply of oxygen to the injured liver [1]. Administration of NAC during acetaminophen overdose acts to rapidly increase GSH synthesis in the liver and reduce mitochondrial protein binding [28]. The amount of NAC required to counteract acetaminophen toxicity is determined by plotting the concentration of acetaminophen in plasma against the time post-overdose on a nomogram [28]. Initiating NAC treatment within 8 h of overdosing minimizes the risk of hepatocellular damage as the effectiveness of NAC treatment is negatively correlated with the time post-overdose [28]. Reports have shown that oral NAC is just as effective as intravenous NAC in treating acetaminophen toxicity within 10 h of overdosing [28]. However, it is important to note that the absorption of orally administered NAC will be hindered when administered following the use of activated charcoal, which is commonly used to mitigate acetaminophen toxicity; as such, the preferred route of administration is intravenous NAC [28].

5.1.2. Non-Acetaminophen-Induced Acute Liver Failure

Etiological agents of non-acetaminophen-induced acute liver failure can include viruses, drugs, toxins, herbal and traditional medicines, and autoimmune-mediated conditions [45]. Immediate intravenous administration of NAC in cases of non-acetaminophen-induced liver failure has been found to reduce mortality, encephalopathy, hospitalization, admission to the ICU, organ failure, and the need for liver transplantation [1].

5.1.3. Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD refers to a condition in which an excessive amount of fat is stored in hepatic cells [46]. Under normal physiological conditions, the liver stores small amounts of energy in the form of the carbohydrate glycogen [46]. Therefore, a healthy liver should contain

little to no fat droplets. Livers that contain fat droplets in more than 5% of hepatic cells are abnormal or pathological [46]. The accumulation of fat in the hepatocytes of NAFLD patients is generally due to a combination of excessive calorie intake and a sedentary lifestyle [46]. However, diabetic patients, particularly type 2 diabetics, and those with abnormal levels of blood lipids or hypertension are also at risk of developing NAFLD [46]. Regardless of the etiological factor(s) leading to the onset of NAFLD, the increased flow of free fatty acids to the liver concurrently increases oxidative stress and suppresses hepatic intracellular antioxidant activity [1].

Experimental studies conducted on the use of NAC in NAFLD patients have demonstrated that NAC can block the accumulation of hepatic lipids and reduce the pro-inflammatory cytokines IL-6, IL-1β, and TNF-∝ and the upstream transcription factor NF-κB [47], which plays a critical role in initiating the inflammatory cascade and immune response related to oxidative stress. NAC has been purported to possess anti-inflammatory properties that include inhibiting activation and translocation of NF-κB, which results in decreased production of TNF-∝, IL-1β, and IL-6 [1]. In a human study conducted by Khoshbaten et al. (2010), it was discovered that oral administration of 600 mg of NAC per 12 h over a 3-month period decreased serum levels of alanine transaminase (ALT) as compared to the group receiving vitamin C [48]. ALT is an enzyme that is predominantly produced by the liver and is commonly used as a biomarker of hepatic inflammation and damage [49].

5.2. Pulmonary Diseases

Inflammation and high levels of oxidative stress coupled with low levels of endogenous antioxidants such as GSH play a critical role in the pathogenesis and progression of pulmonary diseases [1]. As an antioxidant, anti-inflammatory, and mucolytic agent, NAC appears to be a promising therapeutic agent in the treatment of various pulmonary diseases including chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and idiopathic pulmonary fibrosis (IPF) [1] and, most recently, COVID-19 [50] (see Table 2). As a therapeutic agent, NAC is commonly administered orally in the tablet form of 600–1200 mg up to three times a day to treat pulmonary diseases [1]. Studies have shown that dosages of NAC up to 3000 mg/day continue to remain safe and are well-tolerated [1].

5.2.1. Cystic Fibrosis (CF)

CF is a genetic disorder affecting multiple organ systems including the lungs and upper airways, pancreas, liver, intestine, and reproductive organs [51]. The lungs of CF patients produce prolific amounts of viscous mucus, which is difficult to clear. This thick mucus increases susceptibility to recurring chronic infections due to poor expectoration [28]. NAC has been extensively used in the treatment of CF to help improve lung function and eliminate mucus due to its mucolytic properties [1], which are attributed to its cysteine residues that break down the sulfhydryl bridges between glycoproteins in mucus, thereby reducing mucus viscosity [28].

Excessive neutrophil-mediated inflammation in the airways is another key characteristic of CF and is believed to be a cause of lung damage and dysfunction [52]. This uncontrolled inflammation leads to overexposure to reactive oxygen species (ROS) derived from bacteria and/or the activated neutrophils, which in turn further amplifies the inflammation [52] and damages tissues. In addition to being a mucolytic agent, NAC's antioxidant properties may prove to be useful in controlling oxidative stress and excessive inflammation in the airways of CF patients [52]. In a study conducted by Dauletbaev et al. (2009), it was found that a 12-week therapy with a high dose of NAC (2800 mg/day) increased extracellular GSH in the sputum of CF patients; however, NAC treatment did not appear to alter any clinical or inflammatory parameters [52].

5.2.2. Chronic Obstructive Pulmonary Disease (COPD)

COPD refers to a group of chronic progressive lung diseases that cause airflow obstruction and breathing-related issues [53]. COPD is predominantly caused by cigarette smoking;

however, long-term exposure to other lung irritants, such as second-hand smoke, air pollution, chemicals, work-related fumes, and toxic substances can also lead to COPD [53]. While many diseases fall under the umbrella of COPD, chronic bronchitis and emphysema are the two most common types.

The presence of numerous free radicals and oxidants in cigarette smoke contributes to lung inflammation through the induction of oxidative stress. During severe COPD, oxidative stress has been found to be exacerbated and GSH levels further depleted; GSH homeostasis is one of the most important antioxidant defense systems in lung cells [54]. Although inhaled bronchodilators and corticosteroids remain the main treatment for COPD, oxidative stress and inflammation represent promising therapeutic targets for treatment due to their key role in the pathogenesis of COPD [54]. In a study conducted by Messier et al. (2013), NAC was shown to provide protection against injury to murine alveolar type 2 (ATII) cells and lung tissue induced by cigarette smoke [55]. These in vivo and in vitro studies were carried out with mice lacking the nuclear factor erythroid 2-related factor-2 (*Nrf2*), whose gene product is a redox-sensitive transcription factor that is crucial to the regulation of the antioxidant defense system [54,55]. NAC's ability to act as a direct scavenger of free radicals in an Nrf2-independent manner is of considerable importance as Nrf2-dependent endogenous antioxidants are often reduced in COPD patients [54]. In a randomized, placebo-controlled trial conducted by Kasielski and Nowak (2001), in which NAC was administered long-term (12 months), it was found that treatment with 600 mg/day of NAC significantly reduced the concentration of H_2O_2 in the expired breath condensate of stable COPD patients; these patients showed a progressive decrease from baseline H_2O_2 concentrations, reaching statistical significance after 6 months of treatment [56]. After 9 and 12 months of treatment, the concentration of H_2O_2 in the expired breath condensate was 2.3- and 2.6-fold lower, respectively, than in COPD patients in the placebo group. Based on the current data, the Global Initiative for Chronic Obstructive Lung Disease has acknowledged that NAC can be used as an adjunct therapy to help reduce the risk of acute exacerbation of COPD [1].

5.3. Infectious Diseases

NAC's ability to attenuate mediators of oxidative stress and inflammation also makes it a promising treatment or adjuvant for various infectious diseases, such as influenza and COVID-19 (see Table 2).

5.3.1. Influenza

Influenza, also commonly referred to as "the flu", is a contagious respiratory infection caused by various influenza viruses that infect the nose, throat, and lungs [57]. The current standard treatment of severe flu includes early antiviral therapy with a neuraminidase inhibitor, which is associated with improved outcomes in hospitalized seasonal influenza patients [58]. However, a significant number of deaths (~25% mortality) still occur in ICU patients with influenza A (H1N1) despite the use of antivirals [58].

Patients with severe influenza A virus infection (H1N1, H5N1, and H7N9) were found to have high levels of circulating pro-inflammatory cytokines [58]. Due to the significant role that inflammation plays in the pathogenesis of seasonal flu, various studies have been conducted to assess the effectiveness of NAC for the treatment of influenza pneumonia. NAC was found to have indirect anti-viral effects, along with the ability to decrease pro-inflammatory cytokine levels and exert anti-apoptotic activities [59]. NAC can help alleviate the symptoms of influenza, primarily by addressing different mechanisms associated with the infection. However, direct impacts could be because of a reduction in viral load [60]. It has been proposed that NAC might interfere with the ability of the influenza virus to replicate and spread within the body. In human trials, for example, NAC significantly lowered the occurrence of clinically apparent H1N1 influenza disease [61]. Additionally, in cell culture experiments, NAC protected against H3N2 influenza virus-induced oxidative stress, cell death, the expression of inflammatory genes, and NF-κB

activity [62,63]. However, this aspect is still under investigation, and more research is needed to confirm its antiviral properties. High doses of NAC used as an adjuvant treatment in influenza pneumonia patients have also been shown to reduce influenza symptomatology and to improve cell-mediated immunity, which is important for fighting viral infections [61]. NAC's immunomodulatory properties are likely due to its ability to inhibit the activation of oxidant-sensitive pathways, including the NF-κB and p38 mitogen-activated protein kinase (MAPK) signaling pathways [60]. Indeed, cytokine and chemokine levels were significantly reduced following in vivo NAC treatment [60]. Lower numbers of infiltrating macrophages, lymphocytes and neutrophils, and myeloperoxidase (MPO) activity, were also found in influenza-infected lungs following administration (intraperitoneal injection) of NAC in mice [64]. Furthermore, the increased proliferation of influenza-specific lymphocytes and the effector function of cytotoxic lymphocytes were found to be positively correlated with NAC treatment [65]. However, it is important to note that NAC efficacy is dependent on the strain of influenza virus, particularly influenza A virus [59]. It appears that NAC's antioxidant and immunomodulatory properties are more efficacious for highly pathogenic influenza A strains in comparison to low pathogenic influenza A strains [60,66]. While the reasoning behind this strain-dependent variation in efficacy is not yet well understood, it is thought that the differences in terms of how the NF-κB pathway is activated in highly pathogenic versus low pathogenic influenza strains may provide an explanation [59].

5.3.2. COVID-19

COVID-19 deaths are mainly attributed to the acute respiratory distress syndrome (ARDS) associated with SARS-CoV-2 infection, particularly in the elderly and those with co-morbidities [67,68]. There is increasing evidence to indicate that excessive immune activation, and the resulting cytokine storm, are the cause of COVID-19-associated lung injury [67]. This excessive immune activation can be the result of an imbalance in redox homeostasis of which prolonged oxidative stress due to inflammation, increased ROS production, and decreased GSH levels are important factors [67].

NAC has long since been used as an off-label antioxidant; however, there is increasing evidence from both preclinical and clinical studies that NAC can attenuate immune activation and cytokine release, which may be relevant to COVID-19 [50]. In a study conducted by Ungheri et al. (2000), NAC was found to significantly decrease the mortality of influenza-infected mice by reducing the production of ROS and cytokines, such as TNF-∝ and IL-6 [69]. Furthermore, the addition of NAC to the standard treatment protocol for many acute respiratory conditions including influenza, community-acquired pneumonia (CAP), ARDS, and ventilator-associated pneumonia (VAP) was found to decrease the severity of disease by mainly attenuating the immune activation [67].

NAC could be used as a potential therapeutic agent in the treatment of COVID-19 by supporting T-cell responses and modulating inflammation [70]. It might also have a potential application to neutralize the toxicity of the SARS-CoV-2 spike protein both after COVID-19 infection and mRNA-based injection. Almost all SARS-CoV-2 variants have conserved cysteine residues in the spike protein forming disulfide bonds. Upon in silico exposure to NAC, these SARS-CoV-2 cysteine residues conjugate covalently with NAC, which results in perturbation of stereo-specific orientations of spike protein and consequent weakening in the binding affinity of spike protein with ACE2 receptor [71].

Given the major roles that ROS and the cytokine storm play in the pathogenesis of COVID-19, the use of NAC to treat COVID-19 has been proposed [67]. In a small case study (10 cases) conducted by Ibrahim et al. (2020), it was found that the acute-phase protein serum C-reactive protein (CRP) and ferritin levels were decreased in all severe COVID-19 hospitalized patients following twice-daily intravenous administration of 600 mg of NAC [72]; elevated CRP and ferritin levels are indicative of uncontrolled inflammation [73]. Additionally, a significant improvement in liver function and reduced oxygen requirement was also observed in all ten patients enrolled in the Ibrahim et al. (2020) study following the administration of NAC; nine of these patients that previously

required extracorporeal membrane oxygenation (ECMO) showed significant improvement and their ECMO treatment was discontinued after NAC therapy [72]. It is worth noting, considering the recent publication on the potential of immune tolerance induction via IgG4 following COVID-19 booster vaccinations [74], that as early as 1997, De Flora et al. showed that oral administration of NAC (600 mg 2 times/day for 6 months) significantly improved cell-mediated immunity to influenza, shifting the response from tolerogenic to activation in seniors [61]. Although immunological tolerance or energy is appropriate under certain circumstances, such as eliminating reaction to self or food antigens, it can also be inappropriate when it eliminates or limits protective responses to pathogenic agents. The potential to utilize NAC to modulate immunological tolerance is worthy of further investigation.

In summary, clinical studies, as detailed in Table 2, highlight several key findings regarding N-acetylcysteine (NAC). Firstly, NAC stands as a well-established and effective treatment for acetaminophen (paracetamol) overdose. Consistent evidence demonstrates that administering NAC promptly after an overdose can prevent or mitigate liver damage, leading to improved patient outcomes. Secondly, research on NAC's efficacy in chronic obstructive pulmonary disease (COPD) has yielded mixed results. While some studies suggest that NAC may reduce exacerbation frequency and enhance lung function in COPD patients, others have not observed significant benefits. Additionally, NAC serves as a mucolytic agent, aiding individuals with respiratory conditions like cystic fibrosis and chronic bronchitis in clearing mucus from their airways. Clinical investigations affirm its effectiveness in facilitating mucus clearance and enhancing lung function in such cases. Moreover, there is emerging evidence indicating that NAC may reduce oxidative stress, enhance endothelial function, and decrease inflammation, all of which are pertinent to cardiovascular health. Nevertheless, further research is needed to comprehensively understand its impact on cardiovascular outcomes. Furthermore, NAC has been the subject of study for its potential hepatoprotective properties, revealing promising outcomes by lowering liver enzyme levels and enhancing liver function. Lastly, NAC may play a role in alleviating influenza symptoms and aiding recovery, primarily due to its antioxidant and immunomodulatory characteristics.

6. Autoimmune Diseases

Autoimmune diseases encompass a wide variety of illnesses and occur when self-constituents are attacked by a hyperactive immune system [75,76]. Due to a loss of immunological self-tolerance, immune cells begin to attack self-molecules manifesting as an autoimmune response [75]. Inflammatory bowel diseases (IBD) are autoimmune diseases that cause chronic inflammation of the gastrointestinal tract, and they are categorized into two main types, UC and Crohn's diseases [76]. Autoimmune diseases are becoming increasingly prevalent, and their corresponding treatments can result in further immunosuppression, which can lead to systemic infections that potentially cause death [75].

More natural treatment remedies, such as NAC, have been explored for treating IBD [77]. Studies conducted by Ebrahimi et al. (2008) measured certain biomarkers in colon cells in a mouse model to examine the effects of NAC on IBD. NAC was administered at varying amounts (106, 160, and 240 mg/kg) over the course of four days after the induction of colitis, and it was determined that NAC was able to attenuate lipid peroxides, the cytokine TNF-α, and nitric oxides [77]. The researchers concluded that cellular biomarkers for IBD improved with the use of moderate to high doses of NAC [77]. Additionally, research on human UC by Shirazi et al. (2021) examined NAC as an antioxidant agent for treating flare-ups of the illness [78]. In a double-blind controlled clinical trial, patients received 800 mg of NAC or placebo over the course of 16 weeks [78]. The results of the study found significant differences between the two treatment groups, where the NAC-treated patients had fewer incidences of endoscopic relapse compared to the placebo group [78]. Additionally, serum CRP levels, mean fecal calprotectin, and the serum erythrocyte sedimentation rate were lower in the NAC group than in the placebo group [78]. These findings elucidated

the positive effects that NAC has on the treatment of UC [78]. Further studies regarding the protective effects of NAC in treating IBD should be explored in humans seeing as it was proven in mice that NAC can improve cellular biomarkers of IBD disease and elicit positive effects in the treatment of UC within humans [78].

7. Cardiovascular Diseases

Recently, the role of NAC in cardiovascular diseases has also received wide attention from the research community. It has been found that NAC can effectively inhibit myocardial cell apoptosis caused by ischemia-reperfusion injury (IRI) and improve cardiac function [79]. NAC may have an indirect effect on the levels of low-density lipoprotein (LDL) and oxidized LDL, primarily through its antioxidant and anti-inflammatory properties. By increasing GTH levels, NAC helps reduce oxidative stress and the formation of reactive oxygen species (ROS). High oxidative stress can lead to the oxidation of LDL cholesterol, transforming it into oxidized LDL, which is more atherogenic. By reducing oxidative stress, NAC may help inhibit the formation of oxidized LDL [80,81]. It is also reported that NAC may improve the function of endothelial cells lining blood vessels [82]. When the endothelium is healthy, it produces nitric oxide, which helps to relax blood vessels and regulate blood flow. Improved endothelial function can contribute to a better balance of LDL and high-density lipoprotein (HDL) cholesterol. NAC may have these potential benefits, but its impact on LDL and oxidized LDL levels may vary among individuals. The research in this area is still evolving, and the effects of NAC on cholesterol and lipoprotein profiles may be influenced by various factors not discussed here.

8. Chronic Conditions

8.1. Atopic Dermatitis

Atopic dermatitis is a chronic relapsing inflammatory skin disease that is associated with epidermal barrier dysfunction [83]. The most common symptoms patients experience are chronic pruritus and eczematous lesions, which can adversely impact the quality of life for individuals afflicted by this disease [84]. In more recent years, drug therapies targeting the type 2 antibody-mediated immune response have shown a decrease in signs and symptoms of atopic dermatitis; however, the exorbitant expenses of these pharmaceutical agents may restrict their prolonged usage [84]. The use of NAC, which is relatively safe and inexpensive, as an alternative therapeutic agent to these more expensive drugs shows promising results for treating atopic dermatitis [83,85].

Clinical effects of topical NAC for treating dermatitis have proven to increase skin hydration in patients suffering from atopic dermatitis [83]. By measuring skin hydration and trans-epidermal water loss, it was determined that NAC had the ability to reduce oxidative stress and allowed for the restoration of adhesion molecules involved in forming the skin barrier, leading to increased skin hydration in patients with atopic dermatitis [83]. Future research is required to determine the molecular pathways NAC plays in restoring the expression of these adhesion molecules and if NAC would be a suitable candidate for treating other skin ailments such as psoriasis [83].

8.2. Diabetes Mellitus

The prevalence of type 2 diabetes, a multifactorial disease characterized by progressive deterioration of insulin secretion and action, is on the rise and accounts for more than 90% of individuals diagnosed with diabetes [86]. Diabetes is primarily due to insulin resistance, and while the pathogenesis of insulin resistance is not yet clear, oxidative stress, innate immune system activation, and abnormal lipid and/or energy metabolism are considered to play key roles [86].

Several clinical studies have been conducted on the use of NAC as a potential therapeutic agent for insulin resistance and type 2 diabetes [28] (Table 1). In a study conducted by Ribeiro et al. (2011), it was discovered that NAC exhibited beneficial modulatory action on oxidative stress biomarkers in alloxan-induced diabetic rats [87]. In another study con-

ducted by Kaneto et al. (2001), NAC was shown to exert protective effects on the pancreatic β cells of diabetic db/db mice [88]. In this study, prior to NAC treatment, hyperglycemia episodes in mice led to decreased insulin content and insulin gene expression. However, following treatment with NAC, insulin content and insulin mRNA expression were found to be preserved, and the binding of the nuclear factor pancreatic-duodenal homeobox-1 (PDX-1) to insulin was also restored. Furthermore, studies involving diabetic patients demonstrated that the administration of intravenous NAC during hyperglycemic clamp, which measures insulin secretion and pancreatic-cell function, was shown to improve insulin sensitivity and increase peripheral glucose uptake [89].

Table 2. N-acetylcysteine clinical studies.

Disease	Study Type	Study Phase	Dose	Treatment Duration	Administration Routes
Liver diseases					
Acute acetaminophen overdose	Interventional (Clinical Trial) NCT03679442	Phase 1	Dose corresponding to the clinical treatment guidelines for acetaminophen overdosed patients	16 h	Intravenous
Non-alcoholic fatty liver disease	Interventional (Clinical Trial) NCT02117700	Phase 2	600 mg twice/ day	16 weeks	Oral
Pulmonary diseases					
Cystic fibrosis	Interventional (Clinical Trial) NCT00809094	Phase 2	900 mg twice/day	24 weeks	Oral
Chronic Obstructive Pulmonary Disease	Interventional (Clinical Trial) NCT01136239	Phase 4	600 mg twice/ day	One year	Oral
	Interventional (Clinical Trial) NCT00969904	Phase 4	600 mg twice/ day	12 weeks	Oral
	Interventional (Clinical Trial) NCT03388853	Phase 4	1200 mg once daily	4 weeks	Oral
	Interventional (Clinical Trial) NCT02579772	Phase 4	600 mg three times/ day for 4 days prior to experimental procedures and 600 mg on the day of the experiment	4 days	Oral
	Interventional (Clinical Trial) NCT00184977	Phase 4	600 mg once daily	3 years	Oral

Table 2. *Cont.*

Disease	Study Type	Study Phase	Dose	Treatment Duration	Administration Routes
Infectious Diseases					
Influenza	Interventional (Clinical Trial) NCT03900988	Phase 4	100 mg/kg daily as a continuous IV infusion over 24 h	28 days	Intravenous
COVID-19	Interventional (Clinical Trial) NCT04374461	Phase 2	6 g/day	Patients will receive treatment for a max of 3 weeks	Intravenous
	Interventional (Clinical Trial) NCT04928495	Phase 3	1800 mg once daily	10 days	Oral
	Interventional (Clinical Trial) NCT04900129	Phase 1	1.2 g twice/day	One month	Inhalation
	Interventional (Clinical Trial) NCT04792021	Phase 3	600 mg/day	Two weeks/ until hospital discharge or death	Oral
	Interventional (Clinical Trial) NCT04419025	Phase 2	**Inpatients**: 25 mg/kg (rounded up to the nearest 600 mg) every 4 h until discharge and then 1200 mg twice daily × 1 week post-discharge **Outpatients**: 2400 mg × 1 then 1200 mg twice daily × 2 weeks	**Inpatients**: until 1 week post-discharge **Outpatients**: 15 days	Oral
	Interventional (Clinical Trial) NCT04455243	Phase 3	150 mg/kg every 12 h diluted in 200 mL diluent (D5%, NS)	14 days	Oral or intravenous
Diabetes Mellitus	Interventional (Clinical Trial) NCT02206152	Phase 1 and 2	150 mg/kg loading dose over the first hour and then follow that with a 50 mg/kg maintenance dose infused over the next 4 h during a controlled hyperinsulinemic hypoglycemic insulin clamp	Two 2-day treatments separated by 8 weeks	Intravenous
	Interventional (Clinical Trial) NCT01394510	N/A	600 mg twice daily × 2 weeks, then 1200 mg twice daily × 2 weeks	4 weeks	Oral
	Interventional (Clinical Trial) NCT04531163	Phase 2 and 3	1200 mg/day	2 months	Oral
	Interventional (Clinical Trial) NCT00556465	Phase 2 and 3	600 mg twice/ day	3 months	Oral

9. Use of NAC in Domesticated Animal Health and Production

Intestinal integrity is essential to normal physiological function as it is critically involved in nutrition, metabolism, and whole-body homeostasis [10]. Damage to the mucosal epithelium impairs nutrient absorption and compromises immunity. Consequently, this reduces animal growth performance and compromises animal health [10]. Animal stressors, such as early weaning and infection, result in injury and dysfunction of the intestinal mucosal barrier [10], which serves as the first line of defense against endogenous and exogenous microbes and their toxins [10]. While the use of NAC for domesticated animals is not widespread, or common, there is substantial evidence to support its use, particularly during the weaning period to help increase average daily gain, increase food intake, and improve growth performance of neonates. Many nutrients have recently been reported to improve immune function, particularly during stress [90], and, therefore, it is not surprising that NAC may also be considered an immunoceutical because of its immunomodulatory properties.

10. Swine

There is increasing evidence that dietary supplementation of NAC may improve the intestinal morphology and function of livestock species, particularly piglets [10,91]. Indeed, recent studies have found that administration of NAC reduces inflammation, alleviates oxidative stress, improves energy status, and ameliorates intestinal tissue damage in piglets that were immune challenged with bacterial LPS [10]. In this study, dietary supplementation with 500 mg/kg NAC was shown to improve the intestinal histological morphology of LPS-challenged newly weaned piglets by preventing LPS-mediated enhancement of crypt depth, reductions of villus height, and villus height to crypt depth ratio; all of which are indicators of potential intestinal absorption capacity and health. Furthermore, these authors also demonstrated that dietary NAC supplementation also alleviated LPS-mediated reductions of diamine oxidase (DAO) activity in the small intestinal mucosa and enhancement of DAO activity in the plasma; DAO is present in the intestinal mucosa, which is particularly abundant in rapidly dividing cells. DAO activity is a marker of intestinal mucosal maturation and integrity, as well as of mucosal injury and recovery, whereas plasma DAO provides an indication of the extent of mucosal injury. As such, it is evident that NAC is also capable of supporting mucosal barrier function under inflammatory conditions. As an antioxidant agent, NAC can also attenuate the adverse effects of intestinal oxidative stress caused by LPS. For example, Hao et al. (2021) also showed that dietary supplementation of NAC increased the activity of the endogenous antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), ultimately enhancing the antioxidative capacity of the jejunal mucosa, where a significant decrease in malondialdehyde (MDA; biomarker of oxidative stress), H_2O_2, and $O_2^{\bullet -}$ content were observed, along with increased content of GSH [92].

10.1. Swine Weaning Disorders

Early weaning stress is associated with both the generation of oxidative stress and changes in the gut microflora of piglets [93]. Factors contributing to post-weaning stress include hierarchy stress, new housing environment, and transition from liquid to solid feed, among many others [94]. Together, these factors negatively impact intestinal development and physiology and the gut microflora and host immunity, which collectively lead to reduced feed intake, poor growth performance, and increased disease susceptibility [92,94]. ROS, such as $O_2^{\bullet -}$, H_2O_2, and OH, are potential toxic by-products for both aerobic and anaerobic gut microbes [93]. Furthermore, oxidative stress induced by weaning stress causes villus atrophy and suppresses the activities of digestive enzymes of weaned piglets [95]. At birth, the piglet gut microbiota is heavily influenced by the sow's milk and is highly populated with lactic acid bacteria. However, the process of weaning significantly reduces the relative abundance of *Lactobacillus* spp., while simultaneously increasing the

abundance of *Clostridium* spp., *Prevotella* spp., *Proteobacteriaceae,* and *Escherichia coli* and decreasing microbial diversity [94]. *E. coli*, an opportunistic enteric pathogen, is known to colonize the intestinal brush border and secrete enterotoxins that impair intestinal functions resulting in diarrhea [93]. The ability of pathogenic bacteria to utilize nutrients unusable to commensal bacteria further compounds pathogen overgrowth, intestinal inflammation, and post-weaning diarrhea [94].

NAC is known to protect cells against oxidative stress and suppress gut tissue damage [96]. In a study conducted by Xu et al. (2014), it was found that providing NAC in diets at a concentration of 500 mg/kg to weaned piglets led to a reduction of intestinal lipid peroxidation and ROS levels, while simultaneously restoring the activity level of endogenous antioxidant enzymes close to that of the normal suckling control group [93]. The reduction of ROS levels and increased activity of antioxidant enzymes observed in the Xu et al. study was thought to be attributed to improved gut redox status and diminished oxidative stress resulting from NAC's direct and indirect antioxidant activities [93]. Furthermore, these authors also demonstrated that the NAC-containing diet altered the composition of the gut microbiota, where beneficial *Lactobacillus* and *Bifodobacterium* counts were increased, while *E. coli* counts were reduced. It appeared that *Lactobacillus* and *Bifodobacterium* counts are positively correlated with the activities of antioxidant enzymes and negatively correlated to MDA, H_2O_2, $OH^{\bullet}$, and NO in this study, whereas *E. coli* was positively correlated with ROS and negatively correlated with the activities of antioxidant enzymes in weaned piglets [93].

The presence of anti-nutritional factors (ANF) in solid feed is another area of concern around weaning, as ANF is known to induce oxidative stress. β-Conglycin (β-CG) is an ANF found in soybeans that causes inflammation, oxidative stress, mucosal barrier dysfunction, enterocyte damage, and diarrhea, and it impairs nutrient absorption in weaned piglets [11]. However, due to the high-quality source of protein content, soybeans are one of the main plant protein sources used in swine diets, despite the presence of β-CG [11]. While heating, pressurizing, fermenting, enzymatically hydrolyzing, and genetically modifying soybeans helps to reduce or inactivate β-CG, these practices are not able to completely mitigate the anti-nutritional properties of β-CG [11]. Given that β-CG induces oxidative stress, NAC may be beneficial in attenuating the adverse effects of β-CG in piglets. In a study conducted by Wang et al. (2021), dietary supplementation of NAC was found to numerically reduce the incidences of diarrhea in β-CG-challenged piglets, as well as concentrations of H_2O_2 (plasma and jejunum) and MDA (jejunum) [11]. Additionally, NAC supplementation to piglets was found to attenuate β-CG depleted enterocyte protein synthesis as evidenced by the increased abundance of intestinal fatty-acid binding protein (iFABP), as well as jejunal occludin and claudin-1 tight junction proteins [11]. Taken together, the administration of NAC may improve the intestinal integrity and function of piglets consuming ANF found in solid feed at weaning.

10.2. Porcine Epidemic Diarrhea (PED)

Porcine epidemic diarrhea virus (PEDV) is the causative agent of PED. PEDV infects the intestine of young pigs leading to acute, severe atrophic enteritis, profound diarrhea, vomiting, extensive dehydration, and high mortality (70–100%) in seronegative neonatal piglets [97]. PEDV infections have been found to increase certain plasma biochemical parameters, including ALT, total protein (TP), albumin (ALB), thyroglobulin (TG), blood urea nitrogen (BUN), chloride (CL), and gamma-glutamyl transferase (GGT) in pigs, which are indicative of systemic inflammation [7]. These authors found that dietary supplementation with NAC alleviated PEDV-induced injury to the small intestine and improved absorptive function, which was evident by the enhanced villus height and surface area, villus height-to-crypt depth ratio, decreased jejunal and ileal crypt depth, and increased plasma D-xylose concentrations, protein concentration, RNA/DNA ratios and protein/DNA ratios, and up-regulated I-FABP and villin expression in the small intestinal mucosa. Supplementation of NAC to PEDV-infected piglets also helped reduce oxidative stress as indicated by the reduction in H_2O_2 concentration in plasma and small intestinal mucosa.

Since the pig is often considered an ideal model for human biomedical research, because of their similar physiology [98], these porcine studies add further support to the reports in humans showing the immunoceutical potential of NAC in various species.

11. Cattle

Inflammation of the udder and teats (mastitis) and respiratory tract, as well as many postpartum reproductive disorders such as metritis and endometritis, are major challenges that the cattle industry continually faces as these are often complex multifactorial diseases [99–101]. Studies involving the use of NAC to treat bovine endometritis and mastitis have shown promising results. For example, in a study conducted by Constantin and Șonea (2018), in which NAC was used to treat bovine endometritis, the clinical cure rate in the NAC group was 77.2% versus 43.4% in the non-NAC group [102]. Furthermore, the NAC group also presented with a higher pregnancy rate of 66.7% versus 54.6% in the non-NAC group. In a study conducted by Yang et al. (2016), NAC was shown to be an important modulator of antibiotic activity against the major bovine mastitis pathogens, including *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *E. coli*, and *Streptococcus agalactiae*; the addition of 10 mM of NAC reduced the minimum inhibitory concentrations (MIC) of penicillin and ampicillin but led to the enhancement of erythromycin and ciprofloxacin's MIC for all tested bacterial strains [9].

Bovine respiratory disease complex (BRD) is a multifactorial disease encompassing a wide range of both viral and bacterial infections, and it remains the major cause of morbidity and death in feedlot cattle [103,104] and veal calves [105]. Decreased immune defenses caused by stressors, including viral pathogens, make cattle more susceptible to infection by existing pathogenic and opportunistic bacteria in the upper respiratory tract [104,106]. Interestingly, studies involving the use of NAC to prevent or attenuate BRD are lacking, but an in vitro study conducted by Lin et al. (2020) revealed that NAC can attenuate apoptosis and autophagy in lung cells, which might be beneficial in cattle, warranting further research [3].

12. Poultry

Several studies have also studied the use of NAC in poultry production, particularly its use in addressing problems caused by aflatoxin B_1 (AFB_1) intoxication and heat and cold stress. In a study conducted by Valdivia et al. (2001), their results suggested that NAC supplementation (800 mg NAC/kg BW per day) helped to mitigate the severity of AFB_1 toxicity as evidenced by the protection against AFB_1-mediated reductions in body weight and liver and renal damage, as well as AFB_1-induced biochemical alterations [107]. In a study conducted by Li et al. (2020), it was found that the supplementation with 0.1% NAC mitigated cold-induced oxidative stress in broilers by increasing the activities of hepatic antioxidant enzymes [108]. Similarly, Yi et al. (2016) demonstrated that dietary supplementation of 1 g/kg of NAC was able to improve the growth performance of heat-stressed broilers, intestinal morphology, and absorptive function, maintain intestinal energy metabolism, and mitigate intestinal oxidative stress [109].

Given the findings of the above studies, NAC appears to be a promising low-cost and safe therapeutic agent that could be more widely used in the livestock industry to address issues and diseases costing the livestock industry millions each year.

13. Companion Animals

13.1. Dogs or Cats

13.1.1. Acetaminophen Toxicosis

Acetaminophen toxicosis is among the 10 most common toxicoses in dogs based on the number of calls that were received at the ASPCA Animal Poison Control Center between 2001 and 2005 [110]. Cats are especially sensitive to the toxic effects of acetaminophen due to a deficiency in a specific high-affinity acetaminophen glucuronyl transferase [111]. Acetaminophen toxicity can result from either a single toxic dose or from repeated cumulative

dosages resulting in methemoglobinemia, hepatotoxicosis, facial and paw edema, depression, weakness, tachypnea, dyspnea, cyanosis, icterus, vomiting, hypothermia, hepatic necrosis, and death in severe cases [112,113]. Clinical signs of toxicosis are not observed in dogs below doses of 100 mg/kg, and acetaminophen is used therapeutically in dogs at doses of 10 mg/kg every 12 h [112]. Hepatotoxicity in dogs is possible at doses that exceed 100 mg/kg, and at 200 mg/kg, methemoglobinemia is possible [112]. Unlike dogs, there is no safe acetaminophen dose for cats [114]. Signs of toxicity have been found at doses as low as 10 mg/kg [115].

Acetaminophen is primarily metabolized as glucuronide and sulfate conjugates in dogs and cats [112]. Due to a deficiency in the hepatic enzyme, glucuronyl transferases, cats form glucuronides with compounds slowly or not at all [116]. As a result of possessing relatively few isoforms of the specific high-affinity acetaminophen glucuronyl transferase, which mediates the conjugation of acetaminophen with glucuronic acid resulting in its elimination, more of the drug is conjugated to sulfates [111]. However, the sulfation pathway also has a finite capacity, which is much lower in cats compared to other species [111]. Once the glucuronide conjugation and sulfation pathways are saturated, excess acetaminophen is oxidized via the cytochrome P450 microsomal enzyme, resulting in the formation of NAPQI, a highly toxic metabolite [111]. Under normal circumstances, NAPQI is inactivated following its conjugation with GSH; however, in acetaminophen toxicosis, GSH stores are rapidly depleted [116]. If inactivated, NAPQI can cause necrosis of hepatic tissue, as well as covalently bind to cellular macromolecules, mediating the conversion of hemoglobin to methemoglobin and inducing the formation of Heinz body [117].

As with humans, the antidote of choice for acetaminophen poisoning in dogs and cats is NAC [110]. NAC works by directly binding to acetaminophen metabolites thereby rendering them inactive and serving as a GSH precursor [118,119]. NAC can also reduce the extent of hepatic injury and methemoglobinemia [118,119]. To treat acetaminophen toxicosis, activated charcoal is first given to absorb acetaminophen [112]. Oral administration of NAC is then given two to three hours following activated charcoal administration, as activated charcoal may absorb NAC if given too early thereby reducing its effectiveness [112]. A 5% NAC solution is administered orally at an initial loading dose of 140 mg/kg, followed by 70 mg/kg every six hours for at least seven doses [120]. Due to its pungent aroma, oral administration of NAC typically causes nausea and vomiting in dogs and cats [121]. While NAC is not labeled for intravenous use, NAC can be given intravenously slowly in life-threatening situations at an initial dose of 140 mg/kg, followed by a maintenance rate of 70 mg/kg every six hours for seven treatments [122]. Each sterile dose is infused over a period of 30 to 60 min through a 0.2 m Millipore filter [122]. It is advised that when NAC is administered intravenously, doses should be given slowly to minimize potential adverse reactions (hypotension, bronchospasm, and flushing) [121].

13.1.2. Infectious Keratitis

Infectious keratitis is the most common ocular disease presented as corneal ulceration (ulcerative keratitis) in dogs in cats [123,124]. Corneal ulcers are frequently the result of trauma and are not always primarily infected; however, they can be rapidly contaminated with bacteria [123–125]. Diagnosis and management of infectious keratitis cases are crucial as they are a potential threat to sight [126–128]. The three most common bacterial organisms responsible for ulcerative keratitis in dogs and cats are *Staphylococcus*, *Pseudomonas*, and *Streptococcus* species [129–135].

Treatment of infectious keratitis involves topical antimicrobial treatment [136]. Due to the wide spectra of activity between antibiotics and the various bacterial strains involved, there is currently no antimicrobial agent available that is effective against all associated pathogens [136]. While some authors advise the use of a combination of different antimicrobial agents to treat this condition, this can potentially lead to reduced efficacy [136–139]. Many antibiotics are known to interfere with each other or even have antagonistic effects [139]. Furthermore, repeated exposure to antibiotics may alter the bacterial ocular

flora composition and favor the colonization of pathogenic bacteria [140]. Normal conjunctival flora has long been suggested to play a crucial role in ocular defense against invasive infections by inhibiting the colonization of pathogenic species [141]. The prognosis for infectious ulcerative keratitis is guarded, with up to 57% of patients requiring surgical interventions even with intensive antimicrobial therapy [142]. Furthermore, with the increasing development of bacterial resistance, there is a need for an alternative substance with antimicrobial properties.

Various studies have demonstrated that NAC possesses antimicrobial activities and is able to disrupt the biofilm formation of different bacterial species in various anatomical sites [143–148]. The bacterial microorganisms associated with infectious ulcerative keratitis in dogs, cats, and humans are also known to form biofilms [149–152]. While NAC is commonly used topically in human ophthalmology to treat corneal wounds, chemical injuries, keratitis, dry eye disease, and meibomian gland dysfunction, studies investigating the effect of NAC on pathogenic bacterial strains causing corneal ulceration in dogs and cats are lacking [136,153]. The study conducted by Walter et al. (2023) appears to be the first study to be conducted to determine the in vitro antimicrobial activity of NAC against common pathogens associated with infectious keratitis in dogs and cats [136]. NAC was observed to have an in vitro antimicrobial effect against all 38 bacterial isolates tested at relatively low concentrations (0.156–0.625%, 1.56–6.25 mg/mL) [136]. Furthermore, all methicillin-resistant *S. pseudintermedius* isolates that were tested in the study were found to be susceptible to 0.312% NAC [136]. Future research is needed to investigate the antimicrobial effect of NAC in vivo in infectious keratitis patients, as in vitro studies do not address factors such as the expected contact time of isolates with NAC when it is applied to the ocular surface. Topical ophthalmic therapeutics typically remain on the ocular surface for 5–10 min in dogs and cats before they are cleared through the nasolacrimal duct and spilled over the lower eyelid [154]. Furthermore, dilution of NAC due to tear production is also not accounted for in in vitro studies. Nevertheless, NAC appears to be a promising antimicrobial agent that can reduce or replace the use of topical antibiotics for the treatment of infectious ulcerative keratitis.

13.1.3. Type 1 Diabetes Mellitus

Canine diabetes, like human diabetes, is divided into two types: type 1 diabetes and type 2 diabetes, with the former being insulin-dependent and the latter being non-insulin-dependent [155]. Most canine diabetes cases are type 1 diabetes mellitus, which is a metabolic disease associated with insulin deficiency and often hypercholesterolemia [155,156]. Clinical signs of diabetes in dogs include polydipsia, polyuria, and weight loss [155]. Disease progression is often associated with further complications that can cause multiple organ damage, such as pancreatitis, kidney failure, motor dysfunction, cardiovascular disease, cataracts, hypercholesterolemia, digestive system diseases, and stroke [157]. Furthermore, diabetes has been shown to be closely linked to atherosclerosis due to the exacerbation of inflammatory processes and stimulation of the formation of new blood vessels [158]. Oxidative stress is also involved in the pathogenesis of diabetes mellitus [159].

Several studies have been conducted on the use of NAC in the treatment of canine diabetes mellitus. In the study conducted by Wang et al. (2023), the combination of NAC with insulin in the treatment of dogs with type 1 diabetes was found to be able to stably maintain blood glucose levels within the normal range, slow down the rate of weight loss, effectively reduce liver injury, and correct cholesterol metabolism disorder and thus effectively prevent the development of hypercholesterolemia [155]. Ma et al. (2023) reported similar findings in their study investigating the protective mechanism of NAC in combination with insulin against renal injury in diabetic dogs [160]. The authors reported that the combination of insulin with NAC was able to attenuate renal injury in type 1 diabetic dogs by regulating mitochondrial dynamics and FUNDC1-mediated mitophagy [160]. Huo et al. (2022) demonstrated that the combination of NAC with insulin relieved diabetes mellitus-induced inflammation and pyroptosis hepatic injury via the

NLRP3/NF-κB pathway [161]. Overall, the combination of NAC with insulin appears promising in the treatment of canine type 1 diabetes mellitus.

13.1.4. Parvovirus

Canine parvovirus (CPV) is a highly contagious virus that affects both domestic dogs and wild canids worldwide [162]. While CPV infection can affect all ages, severe infection is most common in puppies between the ages of 6 weeks and 4 months, with higher incidences in animal shelters, pet stores, and breeding kennels [162,163]. CPV affects the gastrointestinal tracts of dogs, preferentially infecting and destroying the rapidly dividing cells of the small-intestinal crypt epithelium [164]. Clinical signs of CPV infection include anorexia, lethargy, vomiting, often hemorrhagic diarrhea, abdominal pain and bloating, and hypothermia, with most deaths occurring within the first 48 to 72 hours following the onset of clinical signs [165]. Due to the lack of effective antiviral therapy, supportive therapy is the only option available [162].

In recent years, oxidative stress was observed to be associated with parvovirus infection, with marked enhancement of reactive oxygen and nitrogenous species, lipid peroxidation, DNA damage, and low antioxidant reserve parvo canine patients [166–168]. With oxidative stress being implicated in the pathogenesis of viral diseases, such as feline coronavirus [169], bovine herpes-virus-1 [170], porcine reproductive and respiratory syndrome [171], and rotavirus [172], emphasis has been given on the use of antioxidants for the management of viral diseases [173–175]. Thus, the incorporation of NAC into the therapeutic regimen against CPV may help to ameliorate the clinical signs of CPV. Indeed, in the study conducted by Gaykwad et al. (2018), NAC treatment of parvo-infected dogs was found to progressively improve the leukocyte, neutrophil, monocyte, and eosinophil counts over time in comparison to parvo-infected dogs that only received supportive treatment [162]. Additionally, NAC treatment was found to significantly improve glutathione S-transferase (GST) activity, as well as decrease nitric oxide and MDA concentrations in plasma on day 3 and day 5 following initiation of treatment compared to the group that only received supportive treatment. The authors evaluated oxidative stress on the basis of GST activity and nitric oxide and MDA concentration in plasma. Chethan et al. (2023) also reported similar results with markedly reduced concentrations of MDA, nitric oxide, and IFABP-2 in CPV-positive dogs supplemented with NAC, resveratrol, and ascorbic acid compared to the control group, which only received supportive therapy [176]. Supplementation with NAC and resveratrol was also found to markedly improve total leukocyte and neutrophil count in CPV-affected dogs. The findings from these studies suggest that NAC represents a potential additional treatment option that should be considered when treating CPV canine patients.

13.1.5. Otitis Externa

Canine otitis externa is the most common, often chronic, disorder affecting the ear canal of dogs and is associated with a high rate of recurrence [177]. In an epidemiological study involving 2012 dogs conducted in 2017, the frequency of otitis externa diagnosis was 15.9%, with a recurrence rate of 24% [178]. Underlying allergic conditions, such as atopic dermatitis or cutaneous adverse food reactions, are often the primary cause of otitis, with secondary bacterial otitis as the complicating perpetuating factor [177,179]. Common bacterial pathogens associated with canine otitis externa include *Staphylococcus pseudintermedius*, *Pseudomonas aeruginosa*, β-haemolytic *Streptococcus* spp., and *Proteus* spp. Currently, commercially available treatments approved to treat otitis externa are limited in variety as the antibacterial agents present in the products are from a limited number of drug classes [179]. Furthermore, these commercial products often contain ototoxic ingredients, such as aminoglycosides, that can result in temporary or permanent hearing loss in dogs [180,181].

NAC's antimicrobial and mucolytic properties, as well as its ability to disrupt biofilm formation, make it a promising potential alternative for the treatment of otitis externa in

dogs. Indeed, in an in vitro study conducted by May et al. (2016), NAC was found to have antimicrobial activity against all twenty-two isolates from canine clinical cases of otitis externa [179]. NAC's minimum inhibitory concentration (MIC) for all tested isolates ranged from 5 to 20 mg/mL. These findings are further corroborated by Son and Bae (2021) and Chan et al. (2019) [143,182]. According to Son and Bae (2021), NAC alone was found to be effective at inhibiting *P. aeruginosa*, which was frequently isolated from canine otitis externa cases [182]. In the study conducted by Chan et al. (2019), NAC was found to be effective against all 110 bacterial and yeast isolates obtained from otitis externa cases with MICs ranging from 2500 to 10,000 g/mL [143]. Studies have also been conducted on the use of NAC in combination with antimicrobials on common canine otitis externa bacterial isolates [177,182]. It appears that NAC interactions with antimicrobials when used against otitis externa bacterial isolates are often indifferent or antagonistic rather than synergistic [177,182].

14. NAC Molecular Mechanisms of Action (MOA)

Despite the vast number of NAC-related publications over the recent decades, a clear MOA and a consensus explanation for NAC's antioxidant and radical scavenging activities remains unclear [16,17,37]. Three major narratives have been proposed to explain the observed effects of NAC: (1) oxidant scavenger, (2) GSH replenishment, and (3) disulfide reductant (Figure 1). However, according to Pedre et al. (2021) and Ezeriņa et al. (2018), these narratives are only applicable under very specific circumstances [16,17]. An alternative MOA is also slowly emerging, involving (4) the sulfane sulfur branch of the NAC metabolism [17]; these per- and polysulfides possess antioxidant and cytoprotective properties, which may explain the observed effects attributed to the NAC [17].

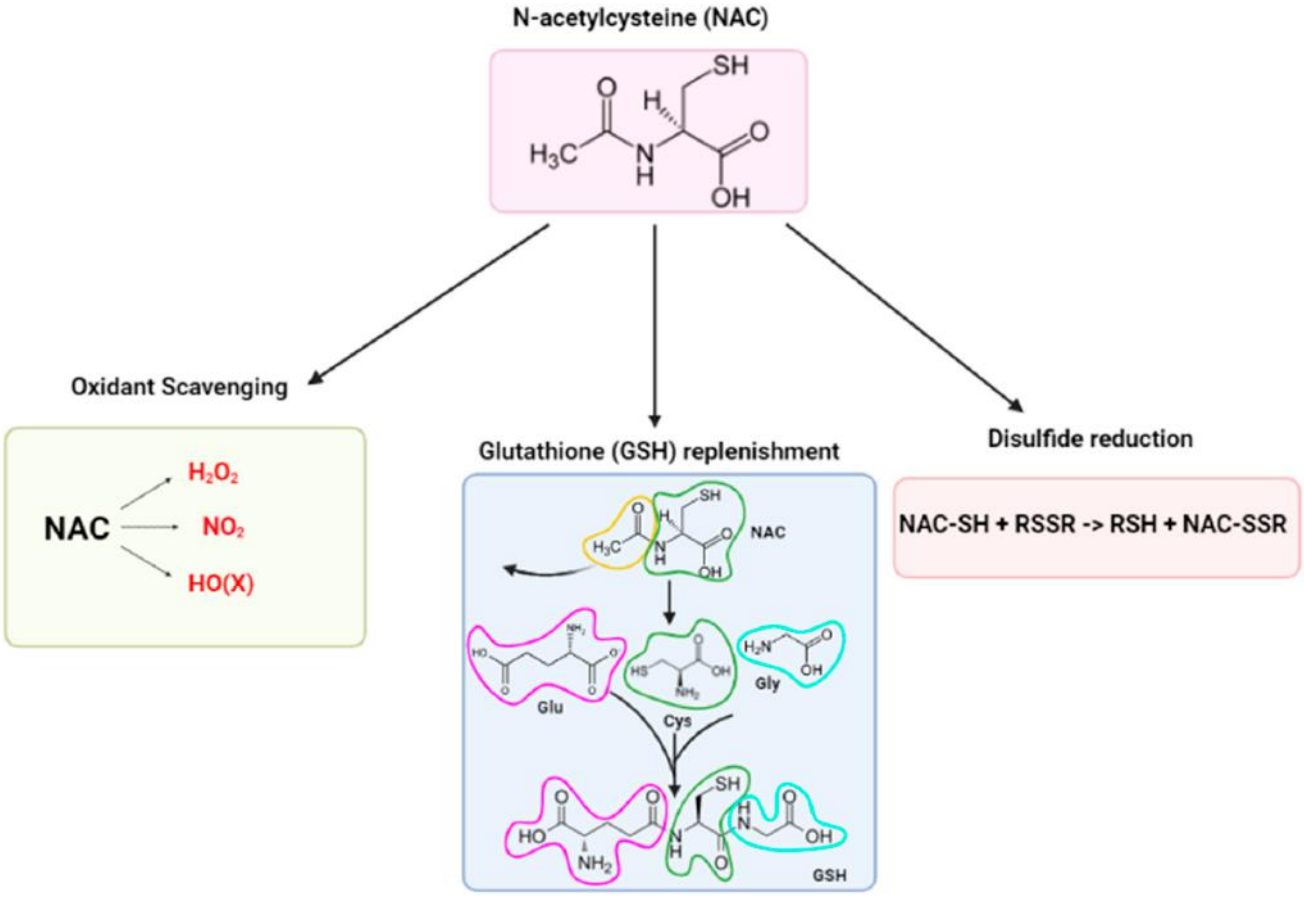

Figure 1. The three major narratives of NAC's mechanisms of action.

(1) Direct antioxidant activity of NAC as an oxygen radical scavenger

For a compound to act as an antioxidant in a biological matrix, its reaction rate with oxidants must be higher than that of endogenous antioxidants and much higher than that of the substrates present [37]. Additionally, the location of ROS generation, the type of ROS produced, and the relative concentration of endogenous antioxidants at the location site must also be taken into consideration when determining the ability of an antioxidant to exert its antioxidant activities [28]. Table 3 provides the reaction rate constants of NAC and other endogenous enzymatic antioxidants with primary oxidant species.

Table 3. Reaction rate constants of N-acetylcysteine (NAC), cysteine (Cys), and glutathione (GSH) towards the oxidant species H_2O_2, $O_2^{\bullet -}$, $HO^{\bullet}$, HO(X), and NO_2.

Oxidant	Antioxidant	K (M^{-1} s^{-1})	References
H_2O_2	NAC	0.16	[183]
	GSH	0.89	[183]
	Cys	2.9	[183]
$O_2^{\bullet -}$	NAC	68	[184]
	GSH	200	[185]
	Cys	15	[184]
$HO^{\bullet}$	NAC	1.36×10^{10}	[186]
	GSH	1.64×10^{10}	[187]
	Cys	$5.35 \pm 0.2 \times 10^{9}$	[188]
HO(X)	NAC	$0.29 \pm 0.04 \times 10^{8}$	[189]
	GSH	$1.2 \pm 0.2 \times 10^{8}$	[189]
	Cys	$3.6 \pm 0.5 \times 10^{8}$	[189]
NO_2	NAC	1×10^{7}	estimated by [190]
	GSH	2×10^{7}	[191]
	Cys	6×10^{7}	[191]

Under physiological conditions, the reaction rate constant of NAC is consistently lower than that of other endogenous enzymatic and non-enzymatic antioxidants, including GSH, cysteine (Cys), and peroxiredoxins [1,37]. Consequently, NAC reactions with primary oxidant species are relatively slow when considering that the concentrations of substrates and endogenous antioxidants are much higher than the concentration of NAC [1,37]. In certain cases, some oxidant species are not targeted by NAC, since the rate of reaction is much too slow to be plausible [37]. These oxidant species include H_2O_2, $O_2^{\bullet -}$, OHNOO, and $HO^{\bullet}$ [37]. However, in situations where the concentration of NAC is higher than that of other thiols (i.e., GSH, Cys), NAC can potentially act on the oxidant species NO_2 and hypohalous acids (HOX) [1,37]. In the case of HOX, NAC can act on HOX because its concentration is higher than that of GSH and Cys in the locality [37]. This situation can be brought on by either pathological conditions or exposure to environmental stressors such as exposure of lung fluids to an inflammatory or oxidative process [37]. Similarly, NAC can also potentially act on NO_2, which is a major component of both indoor and outdoor air pollution and is damaging to the lung epithelium [37].

(2) Indirect antioxidant activity of NAC via glutathione replenishment

In addition to NAC's direct antioxidant activity, NAC also exerts an indirect antioxidant effect through its ability to replenish depleted GSH stores [37]. Most of the antioxidant effects attributed to NAC are the result of increased intracellular GSH [28]. Consequently, certain conditions must be satisfied for NAC to exert its antioxidant activity [28]; the first condition being that the enzymatic machinery required for GSH synthesis is non-defective and expressed at adequate levels, and the second being that GSH levels must be depleted in order for NAC to confer any beneficial effect [28]. Indeed, as demonstrated by Giustarini et al. (2012), neither short-term (5 min) nor long-term (2 weeks) administration of NAC resulted in the elevation of GSH levels in the healthy organs of rats [25]. The lack of

elevation in GSH levels under normal conditions is due to a negative feedback mechanism embedded in the GSH biosynthesis pathway [17].

GSH's poor bioavailability and limited ability to cross phospholipid bilayers make the administration of GSH suboptimal [28]. Similarly, Cys undergoes rapid oxidation to its disulfide moiety upon delivery, thereby generating an inactive disulfide cystine (Cys-Cys), and due to its poor solubility, the sulfhydryl functional group on Cys is rendered temporarily inaccessible [28]. However, acetylating the N-terminal end of Cys, thus creating the compound NAC, increases the stability of the molecule and allows for more efficient delivery of reduced sulfhydryl moieties [28]. While the exact mechanism of how NAC delivers Cys remains unclear, it is postulated that when free intracellular reduced Cys is required for GSH synthesis, intact NAC will permeate the cell membrane before undergoing hydrolysis to yield Cys [28]. The deacetylation of N-acetyl-L-amino acids is catalyzed by aminoacylases I, II, and III [192]; cytosolic acylase I is the aminoacylase responsible for the deacetylation of NAC [37]. Determination of the activity and presence of cytosolic acylase I in various organs of several mammalian species (rat, rabbit, dog, monkey, and man) was carried out by Yamauchi et al. (2002), who concluded that acylase activity was the highest in the kidney of all species studied [193]. These authors found that hepatic cytosolic acylase I activity was 10–22% of that in the kidneys of the rat, rabbit, monkey, and man; however, liver acylase activity in the dog was negligible. Based on these results, the kidney and liver appear to be the main organs responsible for the biotransformation of NAC to the amino acid cysteine in mammals.

(3) NAC as a disulfide reductant

Through the thiol-disulfide interchange mechanism, NAC acts as an efficient reducing agent of protein disulfides [37]. Protein disulfides serve as inter- and intra-subunit crosslinks in secondary and tertiary protein structures, and, as such, play a critical role in maintaining the structure of many proteins, including mucus proteins [194]. Disulfides are also produced through thiol oxidation; a process involved in defense mechanisms against oxidative stress and in redox regulation of cell signaling [194].

The classical thiol-disulfide interchange reaction involves a nucleophilic substitution (S_N2) of a thiol in disulfides with another thiol [194]. This S_N2-type nucleophilic substitution mechanism is a one-step reaction, whereby, in the case of NAC, the thiolate in NAC binds to the central sulfur of the disulfide, thus breaking the disulfide bond and the leaving thiol released via a trisulfide-like transition state structure [37,194]. The rate of the thiol-disulfide interchange reaction is dependent on the nucleophilicity of the thiolate; the higher the nucleophilicity, the greater the reducing ability [37]. The order of S nucleophilicity of the NAC, GSH, and Cys thiols is NAC > GSH > Cys, and, therefore, in comparison to GSH and Cys, NAC has the greatest disulfide-reducing ability [37].

NAC's disulfide-reducing ability is responsible for its mucolytic activity, and, as such, NAC is also considered a mucolytic drug. Mucolytic drugs act on the mucus layer lining the respiratory tract to increase mucosal clearance by reducing the heavily cross-linked mucin polymers, which are the major macromolecular constituents of mucus that are characterized by cysteine-rich domains in their N and C termini [37]. The N and C termini mediate the extension of mucin polymers through end-to-end disulfide linkage of mucin monomers [37]. Mucins also contain Cys-rich regions in their internal domains that are prone to forming internal cross-links when oxidized [37]. Under normal physiological conditions, airway mucus gels are lightly cross-linked, which allows for easy transportation up the mucociliary escalator towards the laryngopharynx, where mucus can be swallowed down the esophagus [37]. However, under pathological conditions involving inflammation and oxidative bursts leading to the oxidation of internal Cys thiols, disulfide cross-links are generated between internal Cys domains rendering the mucus to be heavily crosslinked [37]. Heavily cross-linked mucus cannot be easily transported by the mucociliary escalator, and, consequently, mucus accumulation results in airflow obstruction, atelectasis (collapsed lung), and renders the lungs susceptible to microbial infection [37].

15. Alternative MOA via the Sulfane Sulfur Branch of NAC Metabolism

The use of NAC to replenish GSH levels and protect against GSH-depleting xenobiotics is well-supported by the literature and has, therefore, led many studies to conclude that the restoration of GSH levels by NAC is the cause of the observed beneficial health outcome in all situations [17]. However, if this were true, the observed beneficial effect of NAC should disappear when γ-glutamylcysteine ligase, which catalyzes the first step in the GSH biosynthesis pathway, is inhibited by buthionine sulfoximine (BSO) [17]; in the presence of BSO, NAC should not be able to restore GSH levels and, therefore, should not be able to exert any beneficial effects. However, several studies have also observed beneficial effects of NAC despite GSH replenishment being blocked by BSO [195–199]. These findings suggest that there is an alternative mechanism through which NAC exerts its cytoprotective activities that is independent of GSH replenishment.

In recent years, studies have shown that the exposure of cells to NAC results in enhanced endogenous H_2S production [16,200–202]. Elevated H_2S levels within the physiological range (up to ~20 nM) have been linked to a range of cytoprotective effects [203]. At the organism level, H_2S acts as a positive regulator of vasoactivity and provides protection against ischemia-reperfusion injury, while at the cellular level, H_2S releasing agents inhibit apoptosis, enhance mitochondrial bioenergetics, and provide protection against oxidative stress [204–208]. While the exact molecular mechanism by which H_2S exerts its beneficial effects is not yet well understood, three hypotheses have been proposed to explain the observed persulfide-mediated cytoprotection [17]. For the purposes of this review, only the third hypothesis will be discussed, as it provides a link between H_2S and the protective effects of NAC. This hypothesis argues that the observed beneficial effects of H_2S may be the result of sulfane sulfur species, particularly per-(RSSH) and poly-(RSS_nSR) sulfides, which are products of H_2S oxidation [17]. Sulfane sulfur species are mainly generated by sulfide quinone oxidoreductase (SQR), which catalyzes the first step in the metabolism of H_2S to yield a sulfane sulfur metabolite. Persulfides can also be generated through the oxidation of H_2S by cytochrome C or as products of a non-enzymatic process, whereby H_2S reacts with sulfenic acids, which can also be formed under conditions of oxidative stress [17]. Sulfane sulfur species are known to have cytoprotective properties and have been shown to provide protection against the damaging effects of electrophiles [209–211], metals [212], oxidants [16], and cyanide [213,214]. This hypothesis suggests that persulfides act as scavengers of one/two-electron oxidants and electrophiles due to their higher reactivity in comparison to corresponding thiols [215–217]. This higher reactivity can be explained by the $\propto$-effect and the lower pK_a of persulfides in comparison to corresponding thiols [17]; the $\propto$-effect refers to the increased nucleophilicity of an atom due to the presence of an adjacent atom with lone pair electrons [17]. In the case of persulfides, the nucleophilicity of the adjacent persulfide's outer sulfur atom is enhanced due to the presence of the lone pair electrons of the inner sulfur atom [17]. Additionally, persulfides' lower pK_a value increases the proportion of its reactive deprotonated form at physiological pH [202,215]. Persulfides have also been suggested to be highly efficient terminators of radical chain reactions despite the lack of data on the interaction between persulfides and ROS [218].

To summarize, multiple lines of evidence exist to support the alternative narrative that NAC acts as a donor of sulfane sulfur species, which, in turn, exerts cytoprotective effects and enhances cellular reducing capacity. While the disulfide-breaking agent and oxidant scavenger narratives can be used to explain health benefits observed following the restoration of depleted GSH stores by NAC, this only applies to situations characterized by severe GSH depletion, and not to situations independent of GSH biosynthesis. In contrast, the H_2S/sulfane sulfur narrative explains the antioxidative properties of NAC in a GSH-independent manner. Sulfane sulfur species derived from NAC enhance resistance to oxidative stress by modulating protein activity, protecting thiols against oxidative damage and scavenging radicals. This alternative narrative reconciles the antioxidative properties of NAC with its inherent inefficiency as a direct antioxidant. Thus, the discovery that NAC can also exert its antioxidative effects in a GSH-independent manner warrants re-evaluation

of the possible therapeutic use of NAC regarding other pathological conditions unrelated to disturbances in GSH levels. Additional work is, therefore, required to further determine the conditions by which NAC produces sustained low-level H_2S/sulfane sulfur production and to which extent this confers therapeutic effects.

16. Conclusions

NAC's antioxidant and cytoprotective properties make it a promising therapeutic agent for a wide range of conditions in which oxidative stress plays a major role in the onset and progression of the disease. Numerous studies have been conducted on the use of NAC for treating human pathological conditions, ranging from pulmonary and metabolic diseases, and infectious pathogens such as SARS-CoV2. NAC has also been proven beneficial in treating and alleviating ailments of domesticated animals; especially porcine disorders associated with weaning.

Due to NAC's use for treating a wide range of conditions, further studies are required to determine adequate dosages, appropriate routes of administration, and treatment protocols for each condition to ensure that NAC efficiently exerts its antioxidant, cytoprotective, anti-inflammatory, and mucolytic properties. Special consideration must also be given to identifying any cross-reactivity with specific compounds that, when combined with NAC, can cause it to act as a prooxidant, a substance that induces oxidative stress. For example, Solov'eva et al. (2007) recently discovered that the simultaneous addition of NAC with vitamin B_{12b} (hydroxocobalamin) to cell culture produced a cytotoxic effect [219]. Future potential applications of NAC could include its use for the treatment of respiratory diseases across species, including livestock, where respiratory disease is one of the most common and costly factors affecting the livestock industry, particularly the swine, beef, and dairy cattle industries. Respiratory diseases commonly affect these animals and other species during the critical post-weaning period and can have long-lasting effects on health and productivity. NAC's mucolytic, antioxidant, antimicrobial, and anti-inflammatory properties should prove useful in treating and attenuating the negative impacts associated with respiratory disease and other inflammatory conditions encountered in both human and veterinary medicine.

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This is Exhibit Y referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

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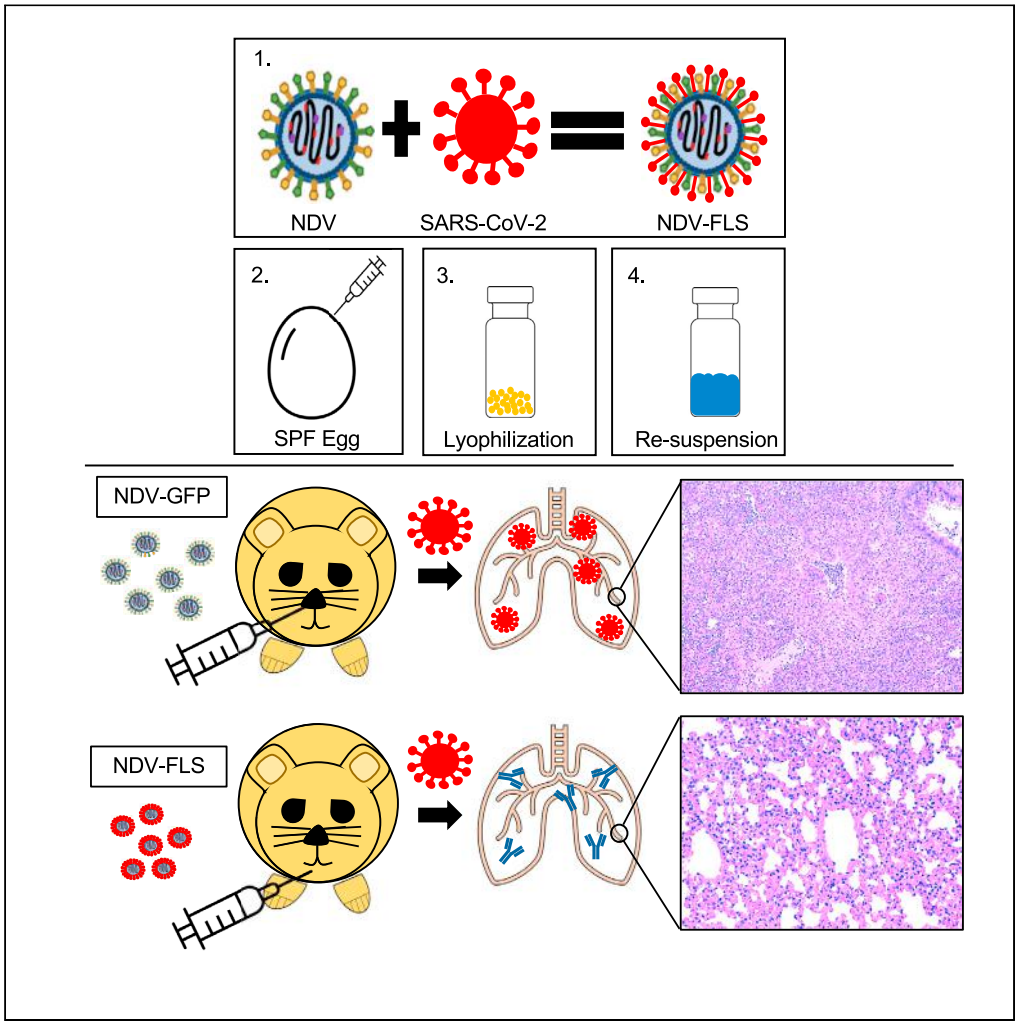
Intranasal vaccination with a Newcastle disease virus-vectored vaccine protects hamsters from SARS-CoV-2 infection and disease



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Highlights

Recombinant NDV passively incorporates SARS-CoV-2 spike into the virion

Vaccination with NDV expressing SARS-CoV-2 spike protects hamsters from disease

No infectious SARS-CoV-2 was detectable in the lungs of vaccinated hamsters

NDV-vectored vaccines represent a viable option for protection against COVID-19

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Intranasal vaccination with a Newcastle disease virus-vectored vaccine protects hamsters from SARS-CoV-2 infection and disease

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SUMMARY

The pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19). Worldwide efforts are being made to develop vaccines to mitigate this pandemic. We engineered two recombinant Newcastle disease virus (NDV) vectors expressing either the full-length SARS-CoV-2 spike protein (NDV-FLS) or a version with a 19 amino acid deletion at the carboxy terminus (NDV-Δ19S). Hamsters receiving two doses (prime-boost) of NDV-FLS developed a robust SARS-CoV-2-neutralizing antibody response, with elimination of infectious virus in the lungs and minimal lung pathology at five days post-challenge. Single-dose vaccination with NDV-FLS significantly reduced SARS-CoV-2 replication in the lungs but only mildly decreased lung inflammation. NDV-Δ19S-treated hamsters had a moderate decrease in SARS-CoV-2 titers in lungs and presented with severe microscopic lesions, suggesting that truncation of the spike protein was a less effective strategy. In summary, NDV-vectored vaccines represent a viable option for protection against COVID-19.

INTRODUCTION

The novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in late 2019 in the city of Wuhan, mainland China, as the causative agent of a severe respiratory disease named *coronavirus disease 2019* (COVID-19) (Andersen et al., 2020; Zhou et al., 2020). The virus has been classified in the *Coronaviridae* family, β-coronavirus genus, and *Sarbecovirus* subgenus (i.e., β-coronavirus subgroup B) (Lu et al., 2020). Phylogenetic analysis has shown that this virus shares ~50% genetic similarity with Middle East respiratory syndrome-CoV, ~80% similarity with SARS-CoV, and >90% similarity with bat β-coronaviruses, suggesting spillover of the virus from bats to humans, possibly through an intermediate adaptive host (El Zowalaty and Jarhult, 2020; Frutos et al., 2020; Lu et al., 2020).

On March 11, 2020, a COVID-19 global pandemic was declared by the World Health Organization (WHO), and by April 2021, the disease had spread worldwide, with over 146 million confirmed cases and more than three million deaths ((WHO), last accessed 2020.09.07). Crude fatality rates have been reported to be around 4% (Karadag, 2020; Verity et al., 2020), although recent estimates that adjusted for demography and case under-ascertainment range between 0.15 and 1.5% (Ioannidis, 2021; Mallapaty, 2020; Russell et al., 2020). The elderly, people with hypertension, immunosuppression, diabetes, and obesity, among other pre-existing conditions, are at a heightened risk of developing severe disease (Williamson et al., 2020).

SARS-CoV-2 is transmitted through respiratory droplets and contact routes (Chan et al., 2020b; Li et al., 2020a; Liu et al., 2020; Ong et al., 2020). In people with severe disease, morbidity and mortality are

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mediated by severe respiratory distress syndrome and vascular disease. The former is caused by diffuse alveolar damage associated with virus replication in type I and II alveolar pneumocytes (Bradley et al., 2020; Calabrese et al., 2020; Martines et al., 2020). Lesions not associated with the respiratory system include endothelial damage, thrombosis, and disseminated intravascular coagulation; however, compelling evidence of virus replication in the endothelium is lacking both in human natural cases or animal models (Besutti et al., 2020; Bradley et al., 2020; Martines et al., 2020; Sia et al., 2020; Varga et al., 2020). Molecular effectors of tissue damage include unchecked production of pro-inflammatory cytokines (i.e., cytokine storm), decreased angiotensin-converting enzyme-2 (ACE2) activity, and activation of a thrombo-inflammatory cascade leading to a hypercoagulable state (Domingo et al., 2020).

Not surprising, multiple research groups have developed vaccine platforms against SARS-CoV-2, including recombinant viral vectors, nucleic acids (DNA, mRNA, and self-replicating RNA), protein subunits, virus-like particles, and live-attenuated or inactivated SARS-CoV-2 virions (Jeyanathan et al., 2020). The vast majority of these vaccines target the SARS-CoV-2 spike (S) protein, the main target antigen for neutralizing antibodies against the virus (Ziegler et al., 2020). By the spring of 2021, in the United States and Canada, two mRNA vaccines, as well as one or two adenovirus-vectored vaccines, respectively, have been approved for emergency use (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). However, it is unclear how these vaccines will be able to curtail the spread of and morbidity caused by novel SARS-CoV-2 variants, such as the B.1.351 (South African variant) and B1.617.2 (Indian/Delta variant), which have been detected since late 2020 (Kirola, 2021) (Kupferschmidt, 2021). Evolution of variants of concerns has highlighted the importance of sterilizing immunity to prevent circulation of SARS-CoV-2 in a partially vaccinated population, an occurrence that can promote development of escape virus mutants due to immunological selective pressure (Peiris and Leung, 2020).

Newcastle disease virus (NDV) has been extensively investigated as a candidate recombinant live vaccine platform for human and veterinary infectious diseases (Kim and Samal, 2016) and shows great potential as a vaccine against SARS-CoV-2 (Shirvani and Samal, 2020). NDV is the type-species of the avian orthoavulavirus-1 (AOaV-1) group, in the *Paramyxoviridae* family (Rima et al., 2019); this virus is enveloped and has a non-segmented, negative-strand RNA genome that allows insertion of foreign genes (up to ~5kb), which are stably expressed at high levels (Zhao et al., 2015). The use of NDV as a candidate vaccine vector in humans offers several advantages. As an avian virus, NDV is antigenically distinct from common human vaccines and pathogens, avoiding the problem of pre-existing immunity that would limit its efficacy in people (Capua and Alexander, 2004). More importantly, as an oncolytic agent NDV has shown an excellent safety profile, whereby direct intravenous, aerosol, or intratumoral administration of large doses of the virus are well tolerated in people (Csatary et al., 1993; Pecora et al., 2002; Wheelock and Dingle, 1964). As a vaccine vector in pre-clinical models, NDV has been shown to be safe and protective in non-human primate models of infection with pathogenic avian influenza virus, Ebola virus, and SARS-CoV (Bukreyev et al., 2005; DiNapoli et al., 2007, 2010). Lastly, NDV is an acute cytoplasmic virus with an encapsidated genome, mitigating concerns about recombination or tissue persistence (Afonso, 2008; Shirvani and Samal, 2020).

In this study, we show the efficacy of an intra-nasally delivered, non-virulent NDV vaccine expressing the SARS-CoV-2 S protein in a Syrian hamster model of COVID-19, by analysis of nasal and lung tissues at the peak of SARS-CoV-2 replication. The use of a non-virulent NDV strain (*i.e.*, lentogenic pathotype which does not cause disease in poultry) circumvents regulatory restriction associated with livestock safety (Cattoli et al., 2011), and the intranasal delivery was aimed at developing mucosal, as well as systemic immunity (Calzas and Chevalier, 2019).

RESULTS

Development of recombinant NDV vectors expressing SARS-CoV-2 spike proteins

In this study, we utilized a fully synthetic molecular clone of lentogenic NDV (LaSota strain, GenBank: AF077761.1) flanked at the 5′ end by a T7 promoter followed by three non-templated G's and at the 3′ by a self-cleaving hepatitis delta virus ribozyme and a T7 terminator sequence (Figure 1A). The ribozyme, by self-cleaving immediately at the end of the viral antigenomic transcript, ensures adherence to the "rule of six" of genomic length (Kolakofsky et al., 1998). The synthetic genome was designed to contain an additional transcriptional cassette between the phosphoprotein (P) and the matrix (M) genes, which was flanked by unique XbaI and MluI restriction sites (to facilitate transgene insertion), and gene start and gene end signals (to promote transcription by the viral polymerase) (Park et al., 2006). An additional L289A mutation

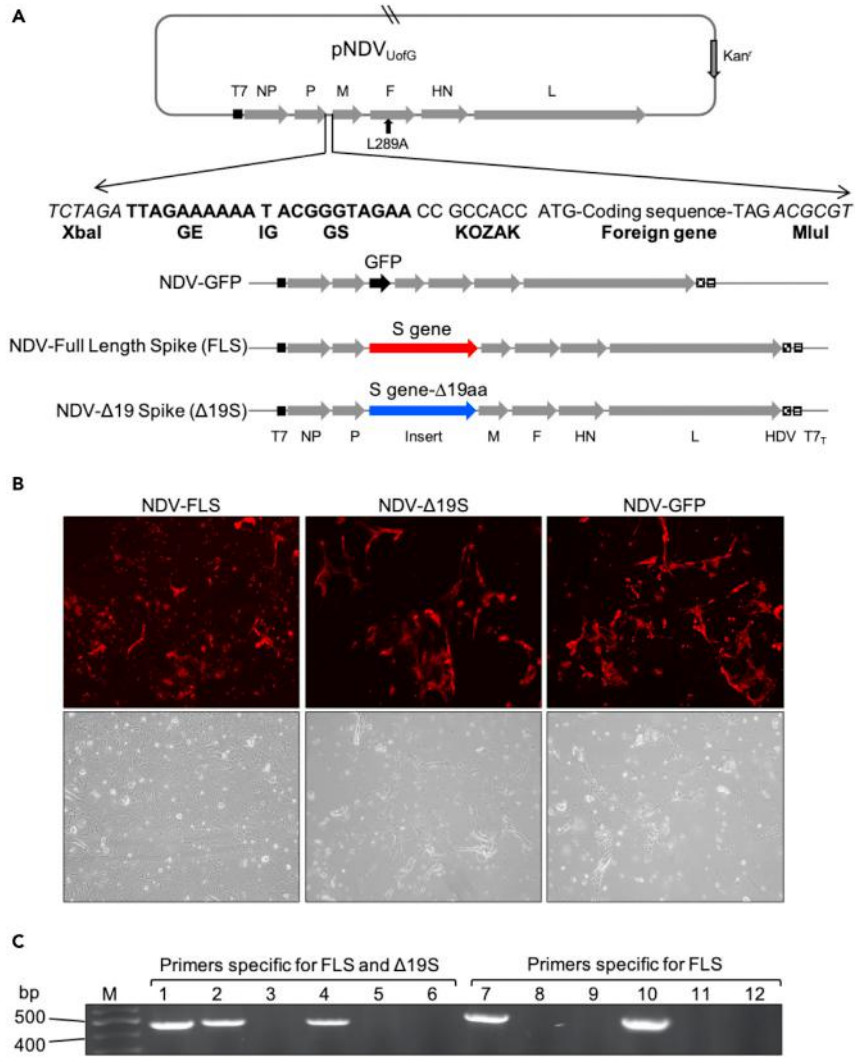

Figure 1. Generation of recombinant NDV vectors expressing the Spike (S) gene from SARS-CoV-2

(A) Schematic representation showing the recombinant NDV genome with XbaI and MluI restriction sites introduced between the P and M genes, where the GFP, full-length spike (FLS) and C-terminal truncated spike (Δ19S) genes were inserted.

(B) Virus replication and cytopathic effect in cells. DF-1 cells were infected with NDV-FLS, NDV-Δ19S, and NDV-GFP virus at a multiplicity of infection (MOI) of 10. The first row shows immunofluorescence (red fluorescence) for NDV nucleoprotein; the second row shows bright field. Both NDV-FLS and NDV-Δ19S replicated in cells, showing expression of NDV nucleoprotein, and caused cytopathic effect similar to the NDV-GFP control.

(C) Agarose gel electrophoresis of PCR-amplified products from DF-1 cells infected with recombinant NDV engineered to express the SARS-CoV-2 S protein. DF-1 cells were infected with either NDV-FLS, NDV-Δ19S, or NDV-GFP. RNA was extracted from cells 12 h later and reverse transcribed to cDNA. Primers were used to target both the FLS and the Δ19S (lanes 1–6); or only the full-length spike (lanes 7–12). (1, 7) NDV-FLS; (2, 8) NDV-Δ19S; (3, 9) NDV-GFP; (4, 10) infectious clone of NDV-full-length spike (positive control); (5, 11) uninfected DF1 (negative control); (6, 12) no-template control. M = GeneRuler 50 bp DNA Ladder (Thermo Fisher Scientific).

in the fusion (F) gene was included to enhance fusogenicity (Sergel et al., 2000). Three recombinant NDV vectors were designed by cloning in a transcriptional cassette expressing either (1) the complete coding sequence of human codon-optimized SARS CoV-2 S protein (NDV-FLS), (2) the partial coding sequence of human codon-optimized spike protein possessing a 19 amino acid deletion from the C-terminus (NDV-Δ19S), and (3) the coding sequence of the enhanced green fluorescent protein (GFP), an immunologically irrelevant protein, to be used as a control (NDV-GFP) (Figure 1A). The truncated version of the spike protein was included as this mutation has been shown to promote more efficient incorporation of spike into

lentiviral particles and vesicular stomatitis virus (Fukushi et al., 2005; Johnson et al., 2020) particles. Successful rescue of recombinant viruses was verified by immunofluorescence staining for the ribonucleoprotein (RNP) complex in NDV-FLS-, NDV-Δ19S-, and NDV-GFP-infected DF-1 chicken fibroblasts (Figure 1B), and by reverse transcription polymerase chain reaction (RT-PCR) to confirm insertion of the spike gene in the viral genome (Figure 1C).

The full-length, but not the Δ19 truncated version, of SARS-CoV-2 spike protein is efficiently incorporated into NDV virions

Western blot analysis of whole cell lysates from DF-1 cells infected at the same multiplicity of infection (MOI = 1) with NDV-FLS or NDV-Δ19S showed robust expression of the full-length S protein at approximately 180 KDa, which was more intensely expressed in NDV-Δ19S- compared with NDV-FLS-infected cells (Figure 2A). Similarly, the cleaved S2 subunit migrated at approximately 100 KDa and was more intensely expressed in NDV-Δ19S- compared with NDV-FLS-infected cells (Figure 2A). To investigate whether the S protein expressed by the NDV vector would be incorporated into the virion and to compare expression with the challenge virus, SARS-CoV-2 and vaccine viruses (NDV-FLS and NDV-Δ19S) were subjected to Western blot analysis. As shown in Figure 2B, SARS-CoV-2 virions incorporated approximately equal amounts of uncleaved and cleaved S protein, as shown by bands right below 200 KDa and one at 100 KDa, respectively, while the virion of the NDV-FLS virus incorporated almost exclusively cleaved S protein. Despite the fact that NDV-Δ19S- infected cells expressed more S protein, this was poorly incorporated into the NDV-Δ19S virions. Nevertheless, S protein expressed from NDV migrated with a similar molecular weight and pattern to that of SARS-CoV-2 S protein.

When incorporated in the NDV virion, the S protein appeared in the cleaved form at around 100 KDa. This suggests efficient cleavage due to the multibasic cleavage site (Ou et al., 2020), a feature that was likely enhanced by the proteolytic activity of the allantoic fluid (Kandeil et al., 2014), as NDV-FLS and NDV-Δ19S were grown in eggs.

Taken together, these data demonstrate that NDV can be engineered to express the SARS-CoV-2 spike protein and that the full-length spike protein is incorporated into the NDV virion more efficiently than the Δ19 truncated version.

NDV vectors expressing SARS-CoV-2 spike proteins do not show an altered infectivity

To test whether the spike protein incorporation into the NDV virion would increase NDV infectivity, we conducted virus neutralization assays in HEK 293T cells over-expressing human ACE2, the receptor for SARS-CoV-2 (Ziegler et al., 2020). Mouse serum, which successfully neutralized lentiviral particles pseudotyped with the S protein in a separate experiment, did not neutralize NDV-FLS or NDV-Δ19S as shown by immunofluorescent staining for NDV RNP at three days post-infection (Figure S2). Instead, immune serum from chickens vaccinated with NDV completely neutralized the viruses (Figure S2). This indicates that incorporation of S protein on the surface of the NDV virion does not alter infectivity or tropism of the vaccine backbone.

To investigate whether expression of the S protein would impact the fusogenic properties of the recombinant NDV vaccines, DF-1 cells were infected with NDV-FLS, NDV-Δ19S, or NDV-GFP, and the number of nuclei was averaged over the total number of cells, with syncytia counted as one cell. As shown in Figure 2C, all three viruses formed syncytia in the presence of trypsin; however, NDV-FLS showed a significantly decreased fusogenic activity compared with either NDV-Δ19S or NDV-GFP (Figure 2D).

Finally, to confirm that the modifications to the NDV vector did not alter its virulence in poultry, we determined the mean death time (MDT) of NDV-FLS, NDV-Δ19S, and NDV-GFP in embryonated chicken eggs. All viruses had an MDT >110 h and thus retained their lentogenic phenotype. Overall, these data demonstrate that NDV engineered to express the SARS-CoV-2 spike protein do not display an altered safety profile *in vitro* and *in ovo*.

NDV vectors expressing SARS-CoV-2 spike proteins are immunogenic in hamsters

We wanted to determine whether vaccination with either the NDV expressing full-length or the truncated spike protein would protect against disease in a hamster model of SARS-CoV-2 infection. Groups of ten Syrian hamsters received intranasal instillations of 10^7 plaque-forming units (PFUs) of either NDV-FLS,

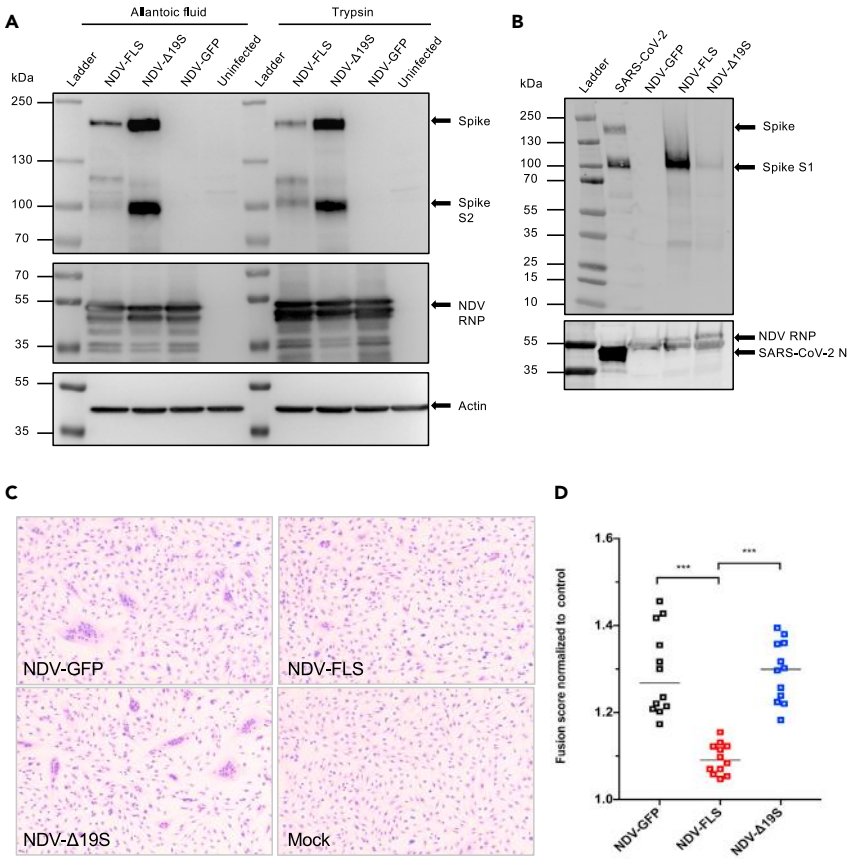

Figure 2. Expression of the spike (S) protein by recombinant NDV vaccines

(A) Western blot analysis of whole cell lysates from DF-1 cells infected with recombinant NDV viruses to confirm S protein expression. Cells were infected with an MOI of 1 with either NDV-FLS, NDV-Δ19S, NDV-GFP, or media only (control), in the presence of allantoic fluid (left) or trypsin (right) for proteolytic activity. Immunoblotting was done 24 h post infection, using rabbit-anti-S2 subunit, mouse-anti-NDV ribonucleoprotein (RNP), and mouse-anti-actin. Full and cleaved S protein (180 KDa and 100 KDa, respectively) are found at much larger amounts in the lysate from NDV-SΔ19-infected cells compared with those infected with NDV-FLS. No S protein expression is observed in cells infected with NDV-GFP or uninfected cells (control). Infection was confirmed by presence of bands corresponding to the RNP of NDV in infected cells.

(B) To compare the incorporation, cleavage, and migration patterns of the S protein between the challenge virus and the vaccine candidates, we conducted Western blot analysis of purified SARS-CoV-2, NDV-FLS, NDV-Δ19S, and NDV-GFP. Equal amounts of purified virus (1.0×10^7 plaque-forming units [PFUs]) were resolved on an SDS-PAGE gel and used for Western blot employing the following primary antibodies: a rabbit anti-S1 (top blot), a rabbit anti-SARS-CoV-2 nucleocapsid, and a mouse anti-NDV RNP (bottom blot). The blot shows incorporation of approximately equal amounts of both full-length and cleaved S protein into the SARS-CoV-2 virion, with bands migrating slightly below 220 KDa and at 100 KDa, respectively. NDV-FLS virions show prominent incorporation of cleaved S protein, while NDV-Δ19S shows significantly less incorporation of cleaved S protein. The NDV-GFP control shows no transgene expression.

(C) For the fusogenicity assay, DF-1 cells were grown in 6-well plates and infected with NDV viruses at an MOI of 0.1. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 2% FBS supplemented with 5% allantoic fluid. 24 hpi media was removed, cells were washed in PBS, fixed with methanol/acetone for 20 min at −20°C, and stained with crystal violet. (D) The fusogenicity score was calculated dividing the number of nuclei by the number of cells in four fields of view per each of the three biological replicates. Counting was assisted using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA). The score for each virus was normalized to the non-infected negative control. Shown are data medians with data scatterplot (n = 3/group). Statistical significance was assessed by using the Kruskal-Wallis and Dunn's test for multiple comparisons. *** = <0.001

NDV-Δ19S, or NDV-GFP (control), as part of a single dose or a two-dose schedule (Figure 3A). At 29 days after the first vaccine dose, low anti-SARS-CoV-2 immunoglobulin (IgG) titers were detected by enzyme-linked immunosorbent assay (ELISA) in the serum of hamsters receiving the NDV-FLS vaccine, while the

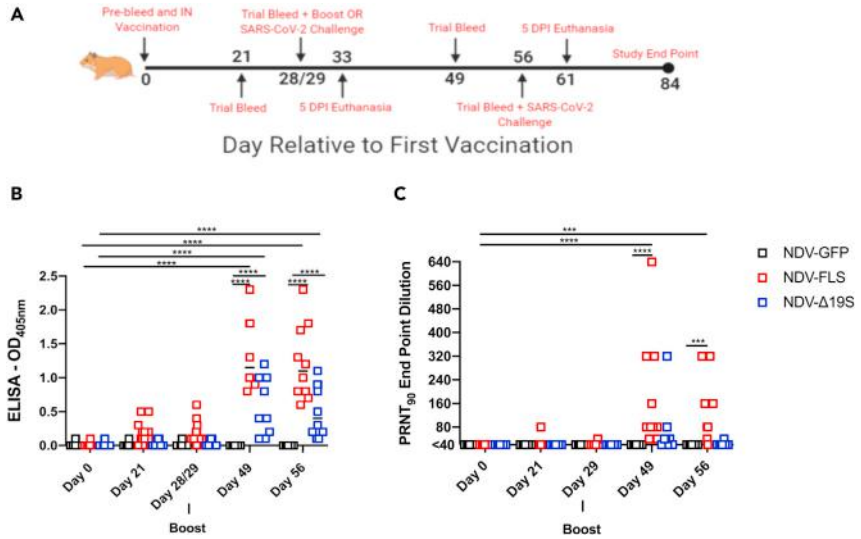

Figure 3. Antibody responses in NDV vaccinated hamsters

Groups of 10 hamsters were vaccinated via intranasal route with 10^7 PFU of NDV expressing either GFP (NDV-GFP), full-length SARS-CoV-2 spike (S) protein (NDV-FLS), or truncated SARS-CoV-2 S protein (NDV-Δ19S), either in a single-dose or a homologous prime-boost schedule 28 days apart. Hamsters were challenged 28 days after prime or boost (A). The concentration of S protein-specific IgG in the serum (diluted 1:400) of vaccinated hamsters was assessed by enzyme-linked immunosorbent assay (ELISA), and is reported as optical density (OD) (B). The levels of neutralizing antibodies in the serum were assessed by plaque reduction neutralization assay 90% ($PRNT_{90}$) (C). Shown are data scatterplots with medians (n = 10/group). Statistical significance was assessed by two-way ANOVA with multiple comparisons. *** = <0.001, **** = <0.0001.

IgG levels in the NDV-Δ19S-treated animals were indistinguishable from those treated with NDV-GFP (Figure 3B). Neutralizing antibodies, detected by plaque reduction neutralization test ($PRNT_{90}$) titers, were only detectable in a single animal in the NDV-FLS group prior to the second vaccine dose (Figure 3C). Following a second homologous vaccine dose, a substantial induction of anti-SARS-CoV-2 humoral immune responses occurred, with a significant increase in serum IgG titers seen in both vaccine groups (Figure 3B), and a significant increase in $PRNT_{90}$ titers in hamsters receiving NDV-FLS (Figure 3C). Only two (of ten) NDV-Δ19S-vaccinated hamsters had $PRNT_{90}$ titers detectable 20 days following the second dose; however, this response waned by the challenge day (Figure 3C). The serum concentration of anti-SARS-CoV-2 IgG and neutralizing titers were significantly higher in the NDV-FLS-treated group, compared to the NDV-Δ19S- and NDV-GFP-treated groups, suggesting that incorporation of the spike protein into the NDV virion may be required for stronger induction of antibody responses. Our data show that a homologous prime-boost vaccination with NDV expressing SARS-CoV-2 spike protein is highly immunogenic in hamsters.

Vaccination of hamsters with NDV vectors expressing SARS-CoV-2 S protects against SARS-CoV-2-induced clinical signs and pathology

To determine whether immune responses in animals receiving a single dose or a homologous prime-boost would result in protection from disease, each of the groups of hamsters described above, which were vaccinated with 10^7 PFU NDV-FLS, NDV-Δ19S, or NDV-GFP, were challenged with 10^5 fifty-percent tissue culture infective dose ($TCID_{50}$) of SARS-CoV-2 on day 56 post-vaccination. An additional experiment was carried out by infecting hamsters at 28 days after receiving the prime only, using the same methodology. Animals were weighed and monitored daily for signs of disease throughout the course of infection. Of ten animals in each group, four were kept for 28 days to examine any differences in weight loss and long-term outcome of infection, while six were euthanized on day five post-infection to examine pathology and viral loads in the tissues during acute infection.

In the single-dose group, NDV-FLS-vaccinated hamsters had significantly less weight loss on day 4 post-infection compared with the NDV-GFP and NDV-Δ19S-vaccinated hamsters. The NDV-Δ19S-vaccinated hamsters showed similar weight loss to controls, including several animals that lost greater than 10% of their initial weight within five days (Figure 4A). In the prime-boost group, the mean weight loss of the

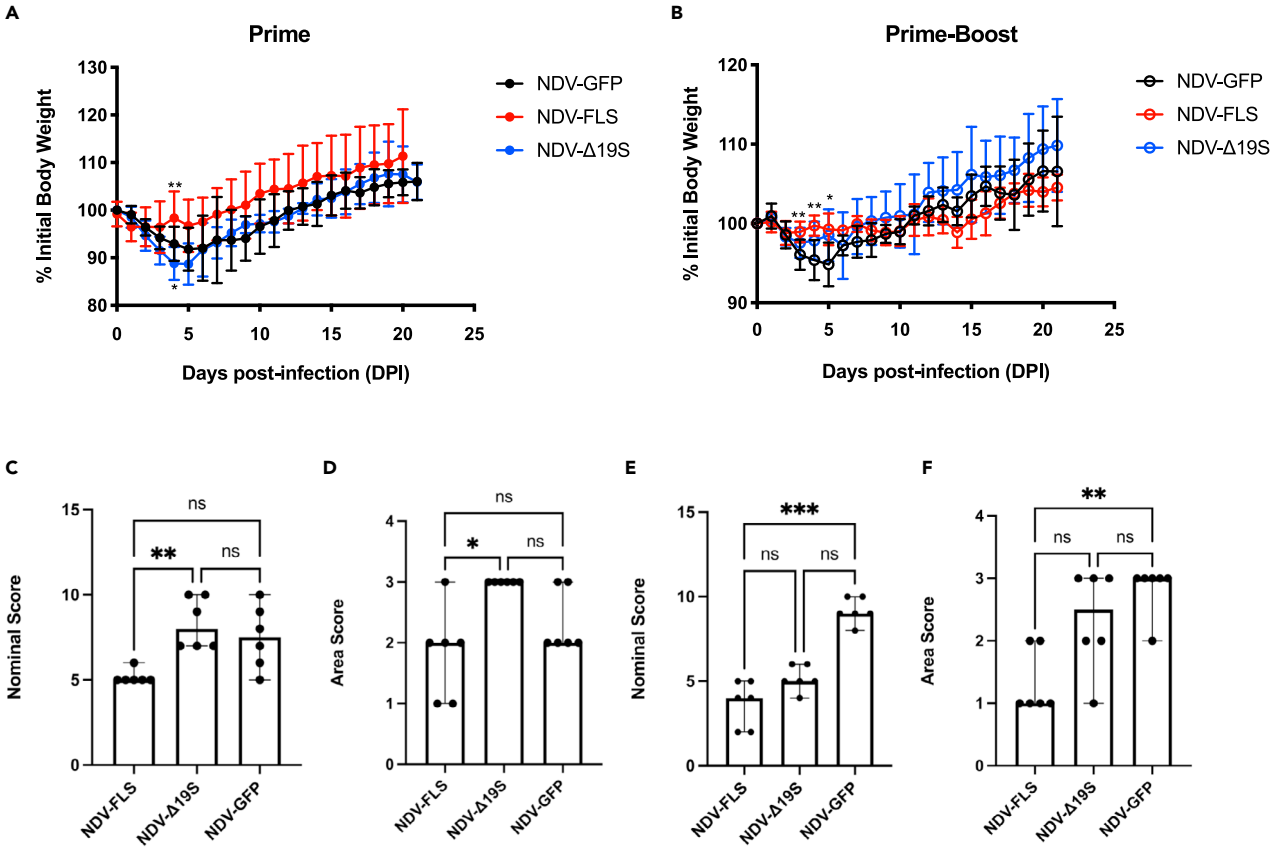

Figure 4. Protective efficacy of NDV vaccines against SARS-CoV-2 infection

Vaccinated hamsters were challenged via intranasal inoculation with 10^5 mean tissue culture infectious dose ($TCID_{50}$) of SARS-CoV-2. Weights of hamsters in the prime (A) or prime-boost (B) schedule were evaluated through day 28 post-challenge (n = 10 or 4/group). At day 5 post-challenge, 6 hamsters per group (n = 6/group) were euthanized and the severity of microscopic lesions for the prime only (C and D) and prime-boost groups (E and F) were assessed. Lesions were evaluated by tallying presence of nominal disease pathology categories (C and E), or by assessing the percentage of affected lung on section (D and F). For graphs A-B, a two-way ANOVA mixed effects model with Tukey's multiple comparison test was run to compare differences in weight between each group at each time point. Circles indicate means. Error bars indicate SD. For graphs C–F, columns represent median +/− range; statistical significance was assessed by Kruskal-Wallis and Dunn's test for multiple comparisons. * = <0.05, ** = <0.01, *** = <0.001.

NDV-FLS-treated hamsters was significantly less than NDV-GFP-treated hamsters on days 3, 4, and 5 post-infection (Figure 4B). Surprisingly, in the NDV-GFP-vaccinated animals, we did not see the weight loss that is typically seen in our hamster model and that has been reported by other groups (Imai et al., 2020). Of the four animals that were not euthanized, none reached greater than 5% weight loss following infection throughout the 28 days, while five of six of the euthanized animals had as high as 8% weight loss and were trending downward. These animals were likely euthanized before reaching peak weight loss, thereby artificially skewing the mean weight loss of that group.

The magnitude of microscopic lesions in the lungs was assessed semi-quantitatively, in order to evaluate the efficacy of the vaccine candidates to decrease the severity of lesions associated with SARS-CoV-2 infection. For both the prime and prime-boost experiments, nominal reporting of lesion categories for each hamster is summarized in Tables S1 and S2, while the extent of affected lung tissue area is reported in Table S3.

Lesions in the control hamsters were similar between the prime only and prime-boost groups (Table S2; see below for a detailed description of histopathology). In the prime only cohort,when the compound score of nominal categories was considered, NDV-FLS-treated hamsters had significantly lower scores compared to the NDV-Δ19S-, but not the NDV-GFP-treated group (Figure 4C). Similarly, NDV-FLS-treated hamsters had

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

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REPLY MOTION RECORD OF THE DEFENDANT DAVID FISMAN - Vol. 2 (Returnable November 19, 2024)

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ONTARIO
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B E T W E E N:

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Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

REPLY MOTION RECORD OF THE DEFENDANT, DAVID FISMAN - Vol. 3

(Returnable November 19, 2024)

March 15, 2023

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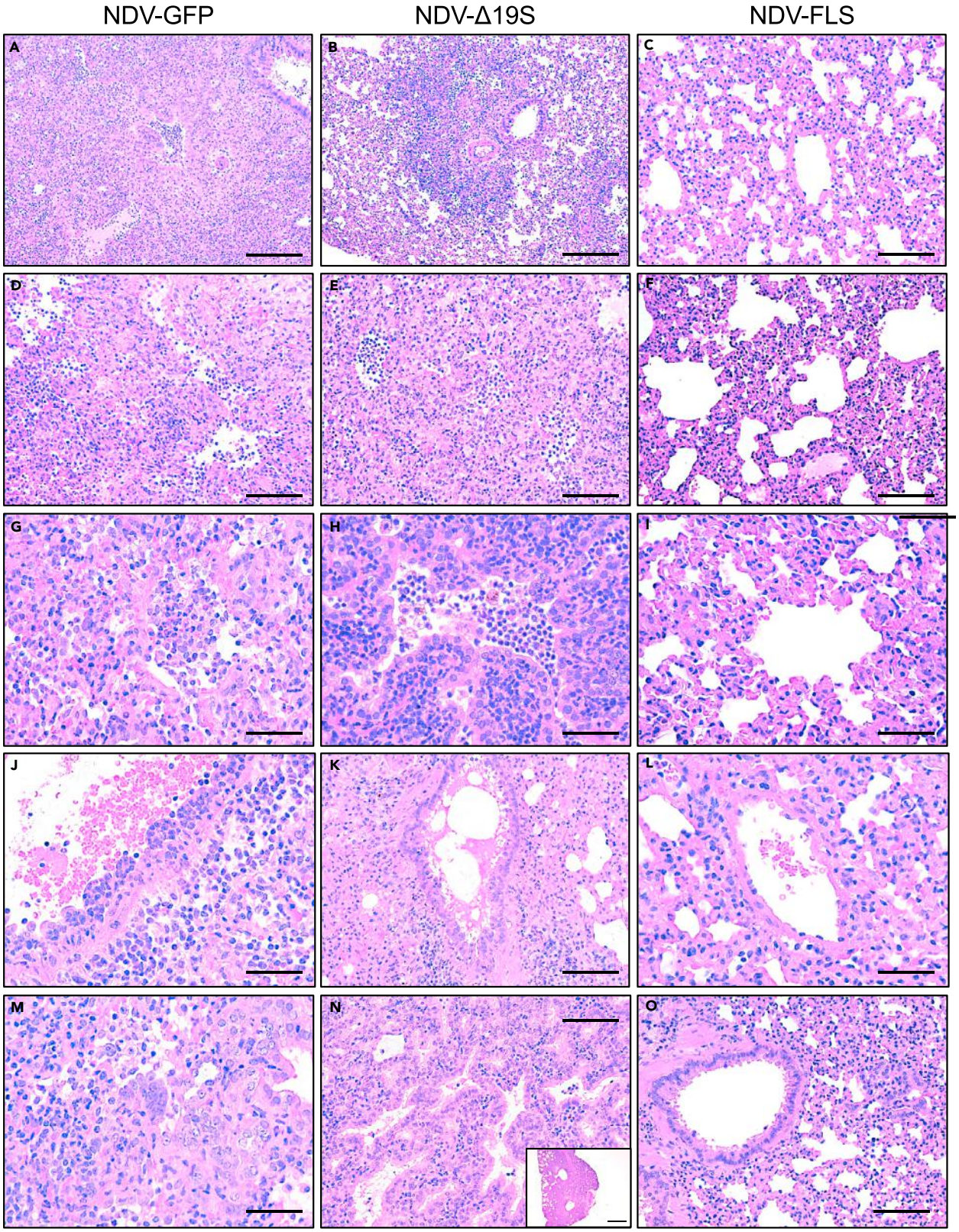

Figure 5. Histological analysis of pulmonary parenchyma from hamsters vaccinated (prime-boost schedule) with NDV-FLS, NDV-Δ19 and NDV-GFP, and challenged with SARS-CoV-2

For each treatment group (NDV-GFP, NDV-Δ19S, and NDV-FLS), the following are showed: inflammation within terminal bronchioles and alveoli (rows 1–3), inflammation around vessels and airways (row 4), and epithelial hyperplasia (row 5). At low magnification, no lesions are observed in the NDV-FLS-treated group (C), while lesions appear multifocal and diffuse in the NDV-Δ19S (B) and NDV-GFP (A) groups, respectively. Exudation of neutrophils, macrophages, and edema fluid is present in NDV-GFP- (D and G) and NDV-Δ19S- (E and H) treated hamsters, while NDV-FLS-treated hamsters showed no lesions (F and I). Perivascular and peribronchiolar inflammation, characterized by edema, mononuclear cells and scattered neutrophils is observed in NDV-GFP (J) and NDV-Δ19S (K) groups but not in the NDV-FLS group (L). Proliferation of mature type II pneumocytes (M) and bronchiolar epithelial cells (N) is observed in both NDV-Δ19S and NDV-GFP groups but not in the NDV-FLS group (O). The inset shows a subgross picture showing an area of marked epithelial hyperplasia. For figures A and B, bar = 200 μm; for figures C-F, K, N, O, bar = 100 μm; for figures G-J, L, M, bar = 50 μm. Inset, bar = 1 mm.

significantly less extensive areas of lung pathology compared with the NDV-Δ19S-, but not the NDV-GFP-treated group (Figure 4D). In the prime-boost cohort, the mean compound nominal score was significantly lower in NDV-FLS-treated hamsters compared to those in the NDV-GFP group; differences were not significant between the scores of the NDV-Δ19S- and the NDV-GFP- or NDV-FLS-treated groups (Figure 4E). Lastly, NDV-GFP- and NDV-Δ19S-treated hamsters had the highest extent of lesions, with five of six and three of six animals showing >50% of lung tissue affected, respectively; while in the NDV-FLS-treated group no hamsters had lesions in >50% of lung sections (Table S3). The average affected area scores were significantly different between NDV-FLS-treated hamsters and those in the NDV-GFP-treated group (Figure 4F).

In the prime-boost experiment, all hamsters treated with NDV-GFP presented with severe exudative lesions characterized by accumulation of sloughed cells, macrophages and neutrophils within the alveolar spaces, variably admixed with multifocal areas of hemorrhage and edema (Figures 5A, 5D, and 5G). In every hamster of this group, most bronchioles were filled with cellular debris and neutrophils. The connective tissues surrounding vessels and bronchioles was markedly expanded by edema and populated by inflammatory cells, such as macrophages, lymphocytes, fewer plasma cells, and scattered neutrophils. In all six hamsters, medium-size vessels showed segmental hyperplasia of the endothelial cells and sub-intimal accumulation of inflammatory cells, although no thrombosis was observed (Figure 5J). As animals were euthanized at day five pi, subacute changes were also observed, which included type II cell hyperplasia (Figure 5M), and presence of hemosiderin-laden macrophages mainly around the terminal bronchioles. In hamsters vaccinated with NDV-Δ19S (Figures 5B, 5E, 5H, 5K, and 5N), lesions were similar to the NDV-GFP-treated group, although the exudative changes were less prominent, noticeably with decreased amounts of desquamated cells in the alveolar spaces and bronchioles. In this group, most hamsters presented with numerous hemosiderin-laden macrophages (suggesting resolving hemorrhage) either in the alveoli or around bronchioles, type II cell hyperplasia (six of six hamsters), and marked hyperplasia of the bronchiolar epithelium in three of six hamsters (Figure 5N). In hamsters vaccinated with NDV-FLS, only two of six hamsters showed mild exudation of neutrophils in the alveoli, and only two presented with multifocal haemorrhages. Most of the lung parenchyma was unaffected (Figures 5C, 5F, 5I, 5L, and 5O). The other changes were subacute, including mild hyperplasia of type II cells (four of six hamsters), , and accumulation of hemosiderin-laden macrophages in four hamsters.

At the time of euthanasia, we also evaluated a series of hematological and serum chemistry parameters to test whether prime-boost vaccination could prevent any hematological changes upon infection of hamsters with SARS-CoV-2. All parameters were within normal limits and no clear trends emerged between vaccinated and control hamsters. Of note, NDV-GFP-vaccinated hamsters showed an elevated neutrophils count, and a higher neutrophil:lymphocyte ratio compared with those that were vaccinated with NDV-FLS, which has been correlated with disease severity in SARS-CoV-2-infected people (Karimi Shahri et al., 2020). This is consistent with the results of microscopic pathology, which showed numerous neutrophils into the lung of NDV-GFP-vaccinated hamsters.

Vaccination of hamsters with NDV vectors expressing SARS-CoV-2 S decreases the magnitude of SARS-CoV-2 replication in tissues

To evaluate the extent of virus replication in tissues, we quantified the presence of SARS-CoV-2 genomic RNA and infectious titers in the tissues of hamsters euthanized on day five pi. In the single-dose group, SARS-CoV-2 genome copy numbers were reduced significantly in the proximal and distal lungs of the NDV-FLS-treated group, while genome copies remained high in the hamsters treated with NDV-Δ19S and were not significantly different compared with controls (Figure 6A). Viral RNA levels in the nasal turbinates, small intestine, and blood did not differ between groups that received a single vaccine dose.

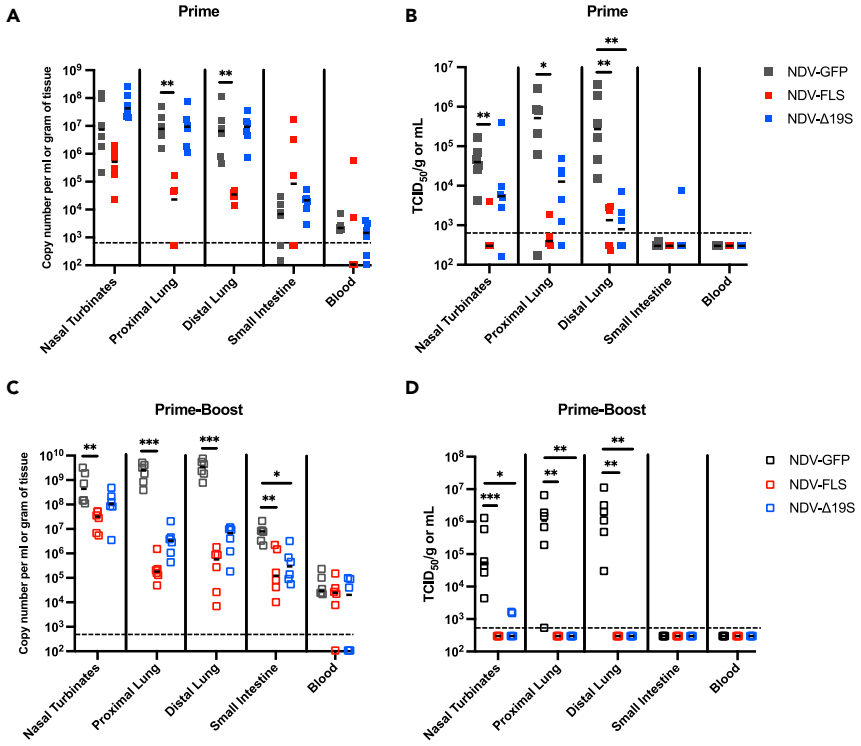

Figure 6. Magnitude of SARS-CoV-2 replication in tissues of vaccinated hamsters

Upon euthanasia at day 5 days post-infection (pi), RNA levels (A and C) and infectious titers (B and D) for SARS-CoV-2 were evaluated in hamsters vaccinated with a prime only (A and B) or prime-boost schedule (C and D). Viral RNA levels are reported as genome copy number, and virus titers are reported as mean tissue culture infectious dose ($TCID_{50}$). Shown are data scatterplot with medians (n = 6/group). The limits of detection are indicated with dashed lines. Differences in the magnitude of virus copy number or infectious titers were assessed by Kruskall-Wallis test with Dunn's test for multiple comparisons. * = <0.05, ** = <0.01, *** = <0.001.

Similarly, a single dose of NDV-FLS significantly reduced the titers of infectious SARS-CoV-2 in the nasal turbinates, proximal, and distal lungs, compared with the NDV-GFP control. The NDV-Δ19S-vaccinated hamsters only had significantly reduced viral titers in the distal lung (Figure 6B). This may be due to some partial protection that prevented the spread of virus into the lower lung.

In animals receiving two vaccine doses, the NDV-FLS group had significantly reduced SARS-CoV-2 genome copies in all tissues examined, except for blood. While the NDV-Δ19S-vaccinated hamsters only had significantly reduced viral RNA levels in the small intestine (Figure 6C), there was a clear trend toward lower viral RNA levels in both the proximal and distal lung (Figure 6C). Hamsters in both prime-boost vaccine groups did not have any infectious virus in the lungs, and only two animals had low levels of virus in the nasal turbinates, suggesting protection of both the upper and lower airway is provided by two vaccine doses (Figure 6D).

We also examined SARS-CoV-2 mucosal shedding following infection with SARS-CoV-2, since a key question regarding the protective efficacy of vaccination and whether vaccination can effectively prevent virus transmission. Oral and rectal swabs were sampled day 2 pi to test whether vaccination may prevent acute virus shedding by these routes. Viral RNA was detected in oral and rectal swabs in all hamsters regardless of the vaccine regimen, without differences in the magnitude of shedding between groups (Figure S1A). Quantification of infectious titers in swabs showed that most hamsters shed at very low levels ($<10^2$ $TCID_{50}$/ml) in both groups, and no differences were observed in the magnitude of shedding (Figure S1B) or the proportion of shedding compared with non-shedding animals (Fisher's exact test, data not show). This suggests that vaccination did not prevent infection and that virus shedding occurred in the early phases of infection, despite protection from disease. Whether the low levels of infectious virus in the oral and rectal swabs are sufficient to infect other hamsters is a question that remains to be answered.

Our data suggest that while vaccination prevented disease and significantly reduced viral growth in the tissues, infected animals may shed virus acutely after infection.

Differential expression of immune response related genes in vaccinated hamsters

To characterize the molecular drivers of inflammation and immune response in vaccinated and non-vaccinated hamsters, we examined the mRNA expression of various immune response-related genes (n = 11) in the lungs of hamsters in the prime-boost experimental groups, at day 5 pi. While several examined genes showed significant difference between vaccinated and control groups, only expression of interleukin (IL)-1β was significantly upregulated in both vaccine groups compared with the control (Figure S3). Vascular endothelial growth factor (VEGF) expression was the other and only gene to be upregulated in the NDV-FLS-treated group compared with the NDV-GFP control (Figure S3). The NDV-Δ19S-treated group showed differential expression of five additional genes compared with the NDV-GFP-treated control group: IL-6, FoxP3, IL-4, transforming growth factor (TGF)-β, and tumor necrosis factor alpha (TNF)-α (Figure S6). Upregulation of cytokine gene expression in vaccinated hamsters may account also for activation of the immune response, rather than tissue damage or inflammation, as pathology shows more severe lesions in the control (NDV-GFP) group.

We also examined relative expression levels of interferon gamma (IFN-γ) and IL-4 to attempt to determine whether a T helper type 1 (Th1) or Th2-associated bias in immune responses between groups may be driving susceptibility to infection. Both vaccine groups had higher, albeit not statistically significant, median IFN-γ:IL-4, ratios, and it is possible that inducing a Th1-associated immune response via vaccination may lead to improved infection outcomes (Jeyanathan et al., 2020).

Lyophilized NDV-FLS retains its infectivity

Given that hamsters vaccinated with NDV-FLS were protected from clinical signs and lesions following SARS-CoV-2 challenge, we sought to investigate whether it would be feasible to lyophilize this promising vaccine candidate thereby greatly simplifying its storage and distribution requirements. Aliquots of NDV-FLS stock containing the same number of PFU were adjusted to a final concentration of 5% sucrose, 5% sucrose/5% iodixanol, or mixed 1:1 with a solution containing 10% lactose, 2% peptone, 10mM Tris-HCl, pH 7.6, and lyophilized for 16h at −52°C. Two days later, samples were reconstituted in phosphate-buffered saline with 5% sucrose at the same volume and virus titer determined. As shown in Figure 7A, there was a ∼2 fold loss of infectivity when NDV-FLS was lyophilized in 10% lactose, 2% peptone, 10mM Tris-HCl, pH 7.6 compared with virus frozen at −70°C. Further, as shown by Western blot analysis, the reconstituted vaccine preparation contained S protein at amounts comparable with the purified virus stock maintained at ultracold temperature, and was able to infect and induce expression of S protein in DF-1 cells (Figure 7B). Given the convenience and greatly simplified storage and transportation requirements of a lyophilized vaccine, further optimization of the lyophilization conditions, as well as assessment of retained efficacy in challenge experiments, is warranted for NDV-based vaccines against SARS-CoV-2.

DISCUSSION

The COVID-19 pandemic has seen an unprecedented number of vaccine candidates, with as many as 14 approved for use in at least one country (Organization). Given the evolution of novel variants of concern (Kirola, 2021; van Oosterhout et al., 2021), the likelihood of persistent spread of SARS-CoV-2 for several years (Scudellari, 2020), emerging data which suggest that the Delta variant may spread more readily than other SARS-CoV-2 variants among people vaccinated against COVID-19 (Brown et al., 2021; Musser et al., 2021; Riemersma et al., 2021), limitations in production capacity of developed vaccines (Khamsi, 2020), and the need for evidence of efficacy for multiple platforms moving forward, there will be a need for a diverse set of vaccine candidates to advance through all stages of development. Here we tested two live vaccine candidates based on an NDV vector expressing SARS-CoV-2 spike protein or a truncated version of the same protein in a hamster model to assess their potential for prevention of COVID-19.

The Syrian hamster has been used by our and other groups as a robust model for SARS-CoV-2 infection, and it is used to examine viral pathogenesis as well as testing vaccine efficacy (Griffin et al., 2021; Muñoz-Fontela et al., 2020). We vaccinated groups of ten hamsters with either NDV vaccine candidate, or NDV expressing GFP as a negative control. The vaccines were administered either as a single dose or as

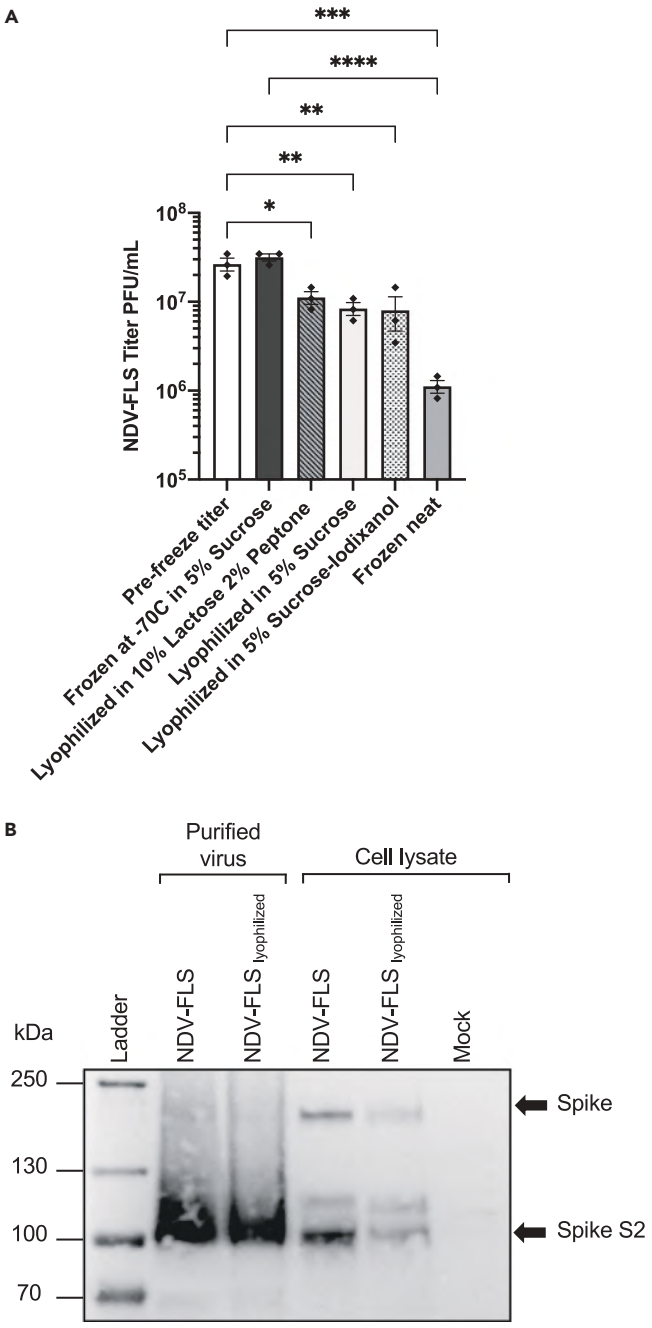

Figure 7. Lyophilized NDV-FLS virus retains infectivity

(A) Triplicate aliquots of equal numbers of NDV-FLS plaque-forming units (PFU) were either immediately frozen at −70°C or adjusted to a final concentration of 5% sucrose, 5% sucrose/5% Iodixanol, or mixed 1:1 with a stabilizing agent comprised of 10% lactose, 2% peptone, 10mM Tris-HCl, pH 7.6 and lyophilized at 44 x 10-3 MBAR and −52°C for 16 h. Lyophilized samples were stored at 4°C for 48 h before being resuspended in 1mL 5% sucrose/PBS and titered by mean tissue culture infectious dose ($TCID_{50}$) in DF-1 cells. Shown are data averages +/− SD (n = 3/group). Statistical analysis was by one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons. * = <0.05, ** = <0.01, *** = <0.001. (B) The lyophilized and reconstituted NDV-FLS (10% lactose and 2% peptone preparation) contains similar levels of Spike (S) protein compared to the purified virus stored at −70°C, as assessed by Western blot analysis when equal amounts of virus are loaded on the gel (10^7 PFU). Lyophilized and reconstituted NDV-FLS (10% lactose and 2% peptone preparation) expresses S protein in DF-1 cells at similar levels compared to NDV-FLS stored at −70°C, as assessed by Western blot of lysates from DF-1 infected with the same multiplicity of infection (MOI = 0.5).

two homologous doses 28 days apart. Both vaccines expressing SARS-CoV-2 S protein were immunogenic as assessed by ELISA for anti-SARS-CoV-2 IgG. Neither vaccine induced significantly high IgG titers after a single dose; however, following homologous booster immunization, both vaccine groups had significantly higher antibody titers compared with controls. When evaluating a prime-boost vaccination schedule, the vaccine expressing full-length S protein induced neutralizing antibody responses that were significantly greater than those seen in the group that received the vaccine with the truncated S protein. It is possible the low incorporation of the Δ19S into the NDV virion might have compromised an effective immune response due to lack of surface antigen presentation, despite the fact that NDV-Δ19S appeared to express higher amounts of S protein *in vitro*. Nonetheless, even the vaccine expressing the full-length S protein induced only modest $PRNT_{90}$ titers, suggesting that efficacy to protect from clinical disease and microscopic pathology may not be entirely dependent upon the magnitude of neutralizing antibody titers. Similarly, hamsters in the prime-boost NDV-Δ19S group appeared to be nearly fully protected from SARS-CoV-2 replication in the lungs, where no infectious titers were evident, despite barely detectable neutralizing antibodies and modest IgG serum titers. It is possible that protection following vaccination with NDV may also rely on T cell responses, which might have been more strongly activated by the NDV-Δ19S vaccine, as it expresses higher level of S protein upon infection. However, this was not examined in our study due to a lack of appropriate reagents, such as hamster-specific antibodies for flow cytometry and depletion studies. Overall, these data suggest that some protection against disease and decreased viral replication can be afforded in the absence of high titers of neutralizing antibodies and further characterization of the immune responses generated by NDV-vectored vaccines, and others will be critical for enhanced immunization strategies.

Treatment with either NDV vaccine was able to decrease or eliminate SARS-CoV-2 replication in the lungs of infected hamsters. Hamsters that received a single vaccine dose had only modest reductions in viral loads in the upper and lower airways but vaccination with NDV-FLS afforded significant decrease in virus titers with only one dose. In the prime-boost cohort, aside from two hamsters with low levels of detectable virus in the nasal turbinates (NDV-Δ19S group), infectious virus could not be detected in the airways of vaccinated hamsters. Consistent with previous findings, there were still high levels of viral RNA in tissues of infected animals, even in the absence of detectable virus. This is likely due to detection of subgenomic mRNA, intermediates of genomic replication, or the presence of intact degraded non-viable viral particles in long-lived phagocytic cells such as macrophages and dendritic cells.

While vaccination with a prime-boost regimen induced near sterilizing immunity in the airways at day 5 pi, detection of viral RNA and infectious virus at day 2 pi in the oral and rectal swabs from all groups (i.e., regardless of regimen) suggests the possibility that vaccination does not eliminate viral shedding during the acute stages of infection. However, on day 5 pi, when we collected tissues rather than swabs, we did not detect any replicating virus in tissues that would lead to shedding suggesting that the vaccine prevented shedding at this time point. This is in line with evidence gathered from the ongoing vaccination campaign, showing that infection and shedding in vaccinated people does occur and is related to the levels of antibody titers in blood, despite still proving highly effective against clinical disease (Bergwerk et al., 2021; Brown et al., 2021). Nonetheless, our data show that vaccination with an NDV vector provides clear protection against SARS-CoV-2 replication and establishment in the airways and lungs.

In the monodose schedule, NDV-FLS-vaccinated hamsters had mildly decreased severity and distribution of microscopic lesions compared with the NDV-Δ19S- and NDV-GFP-treated groups, although differences with the latter were not statistically significant. These results agree with the findings of virus titration in organs, which showed that the vaccines administered as a monodose did not confer sterilizing immunity and still afforded virus replication. In the prime-boost schedule, the severity and extent of microscopic lesions stratified the treatment groups consistently with the magnitude of serum neutralizing antibodies and levels of SARS-CoV-2 replication in lungs. Hamsters vaccinated with NDV-FLS showed no to minimal lesions, some of which (*i.e.*, epithelial hyperplasia and hemosiderophages) suggested that they were in the healing phase of the disease, without the presence of exudate. Hamsters treated with NDV-GFP showed the most severe lesions, characterized by presence of exudate in the alveoli and bronchioles. Finally, hamsters treated with NDV-Δ19S had lesions slightly less severe in extent and severity to those in the NDV-GFP group, albeit these differences were not statistically significant. Taken together, the pathology data indicate that NDV-FLS had a high protective effect against development of SARS-CoV-2-induced lesions, while NDV-Δ19S did so only partially.

Overall, the microscopic lesions caused by SARS-CoV-2 in the hamsters were consistent with the pathology documented elsewhere with this animal model (Chan et al., 2020a; Sia et al., 2020). The lesions observed in the control group (NDV-GFP) are also similar to what we observed in another pathogenesis study conducted by our team using untreated/naive hamsters (Griffin et al., 2021), indicating that the NDV backbone does not significantly affect the severity of SARS-CoV-2 infection in the lung. Lastly, development of hyaline membranes and marked fibrin exudation were not observed in our study. Although this is a typical feature of SARS-CoV-2 pathology in humans, this lesion has not been reported consistently in experimental settings (Gruber et al., 2020). Similarly, our model recapitulated development of vascular damage (*i.e.*, endothelialitis); however no thrombosis was observed (Gruber et al., 2020).

The protection from pulmonary lesions reflects abated clinical signs, as shown by the overall and mean weight loss differences between vaccinated and control hamsters, especially in the single-dose group. In the prime-boost group, while NDV-FLS protected from acute weight loss at 5 dpi, the control group out-paced NDV-FLS-vaccinated hamsters at later time points, up to the end of the experiment (day 28 pi). This could be partly explained by sampling artifact, caused by euthanizing animals with greater clinical signs first, and leaving less affected hamsters for long-term weight assessment. Alternatively, it is possible that hamster suffering a more severe disease (i.e., control group) may undergo a compensatory weight gain phase. This has been also observed by our group in a recent COVID-19 pathogenesis study that used a similar hamster model (Griffin et al., 2021).

Findings from the vaccination experiment unquestionably show that NDV-FLS is superior to NDV-Δ19S as a vaccine candidate against COVID-19. This suggests that incorporation of the vaccine antigen within the envelope of the NDV virion is necessary to trigger an effective immune response, possibly by direct interaction with B cell receptors as a prerequisite for induction of antibody responses (Heesters et al., 2016). While NDV-Δ19S-infected cells expressed higher levels of S protein compared with NDV-FLS-infected cells, the truncated S protein was poorly incorporated in the NDV envelope, as shown by Western blot analysis of purified vaccine preparations. While truncation of the 19 carboxy-terminal amino acids of the S protein has been shown to increase envelope incorporation in other viruses, such as VSV and lentiviral particles (Duan et al., 2020), this was not the case for NDV. This occurrence may be caused by a spatial mismatch between localization of the truncated S protein on the plasma membrane and specific sites of NDV release, which are cholesterol-rich sites defined as lipid rafts (Laliberte et al., 2006). Alternatively, truncation of the S protein may have led to inappropriate interaction with the NDV matrix (M) protein, which binds electrostatically with the NDV surface glycoproteins and drives budding of mature virions (Battisti et al., 2012; García-Sastre et al., 1989). This finding indicates that decoration of the vaccine envelope with the antigen of interest can improve the efficacy of vaccination, and further suggests that for development of NDV-based vaccine platforms, foreign surface epitopes should be chimerized with the transmembrane and cytoplasmic domains of NDV surface proteins (Sun et al., 2020a).

Several biomarkers of disease have been defined in COVID-19 patients, including changes in the concentration of cells in blood and in the presence of various cytokines and vascular growth factors (Karimi Shahri et al., 2020). In this study, we examined whether any significant hematological or serum chemistry changes could be detected and whether these might be indicative of disease progression, compared with protection against disease in prime-boost vaccinated animals. We detected elevated neutrophil counts as well as neutrophil:lymphocyte ratios in the blood of control hamsters, which have been linked to more severe disease progression (Li et al., 2020b). Specifically, neutrophilia appears to be induced by inflammatory mediators produced in diseased tissues, such as lung, and lymphopenia is a typical feature of acute viral infection, such as SARS-CoV-2 (Frater et al., 2020; Karimi Shahri et al., 2020). In the NDV-GFP-treated group, numerous neutrophils were recruited in the areas of affected lung, suggesting that neutrophilia was needed to satisfy peripheral tissue demands during infection. Elevated hemoglobin levels seen in control hamsters could also indicate some level of pulmonary distress and greater need for oxygen throughout the body, although in human patients low levels of hemoglobin appear to be associated with a poor outcome (Karimi Shahri et al., 2020). While some differences were seen in serum biomarkers such as glucose, urea nitrogen, and calcium, along with the above-mentioned changes in hematological markers, it is not possible to interpret the kinetics of these parameters with only one data point available.

Acute SARS-CoV-2 infection in hamsters lasts about one week, with a peak of infection between days two and four, therefore critical questions regarding viral replication and the immune responses generated may require

examination of animals euthanized at multiple time points. Accordingly, our examination of differentially expressed immune-related genes at day 5 pi represents a snapshot of one time point. Overall, our data show a trend by which cytokines appear to be more expressed in the lungs of vaccinated and challenged hamsters, compared to the control group, possibly as an effect of immune response activation. These differences appear to be more obvious in the NDV-Δ19S-vaccinated group, and may reflect the high levels of S protein expression seen *in vitro*. Conceivably, production of large amounts of intracellular foreign epitope may have triggered a more robust cell-mediated response compared to NDV-FLS, which induces lower S protein expression *in vitro* despite incorporating more S protein onto the envelope. Depletion studies could help define the contribution of cell-mediated and humoral response in the immunity elicited by our vaccines, however, lack of suitable reagents in hamsters limited our study to examining antibody responses and cytokine gene expression levels. Further characterization of immune responses and protective efficacy of NDV vaccines in other models such as mice or non-human primates may provide further insights into what immunological effector mechanisms are induced by vaccination with NDV and how these play a role in protection from disease.

Recombinant NDV vaccine platforms provide an option that has proven to be safe and immunogenic in several studies (Bukreyev et al., 2005; DiNapoli et al., 2007, 2010; Kim and Samal, 2016), and here is shown to provide full protection in a hamster model of COVID-19. Overall, the vaccines tested in this study were safe in hamsters, and expression of the S protein did not appear to change the virulence of the backbone. The vaccines were lentogenic (*i.e.*, non-virulent) in chicken eggs, consistent with a non-virulent fusion cleavage site of the NDV fusion protein (Cattoli et al., 2011), and were not neutralized by S protein-specific serum, suggesting that the S protein did not contribute to infectivity of NDV recombinants. Moreover, lyophilization of the NDV-FLS vaccine did not substantially reduce virus titer and maintained its ability to infect cells and express the S protein. Although these are attractive analytical qualities, the lyophilized and reconstituted NDV-FLS should be further tested in vaccination/challenge studies, to conclusively demonstrate retained immunogenicity upon lyophilization.

Despite having substantial incorporation of S protein in the envelope, NDV-FLS had lower fusogenic activity compared with NDV-Δ19S or NDV-GFP. The higher fusogenicity of NDV-Δ19S compared with NDV-FLS is likely mediated by the higher expression of truncated spike protein, as seen in DF-1 cells, and its increased localization on the plasma membrane, due to lack of the Golgi anchoring signal (Boson et al., 2021). Nonetheless, the ability of NDV-Δ19S to develop syncytia was similar to what observed with NDV-GFP, and did not increase virulence for embryonated eggs.

Recently, a live attenuated NDV vaccine against COVID-19 has been developed by another group. This vaccine was engineered to express either a wild-type spike protein, or a stabilized version that does not undergo cleavage (pre-fusion stabilized) (Sun et al., 2020a). Upon intramuscular delivery using 10–50 μg of purified virus preparation (virus was not quantified by an infectivity assay) in a homologous prime-boost schedule 21 days apart, both vaccines induced neutralizing antibody and completely inhibited replication of a mouse-adapted SARS-CoV-2 strain in the lungs of BALB/c mice. While this is similar to what was observed with our NDV-FLS prime-boost group, it should be noted that in mice, titers of SARS-CoV-2 in lung tissues peaked at approximately 10^4 PFU/lung lobe, as opposed to titers of up to 10^7 $TCID_{50}$/g of lung in the control hamsters of our study. Similarly, mice did not develop clinical signs or microscopic lesions, preventing assessment of protection against clinical signs or tissue damage (Sun et al., 2020a). An inactivated version of the same vaccine (10 μg alone or 5 μg with adjuvant) was also administered intramuscularly in an homologous prime-boost regimen, and it protected both mice and hamsters from virus replication in lung tissues at day 5 pi; however, microscopic lung pathology was not assessed in this research (Sun et al., 2020b). While these studies support the notion that NDV is a successful and versatile vaccine platform against SARS-CoV-2, differences regarding vaccine type (inactivated vs. infectious), delivery (intranasal vs. intramuscular), dose assessment (mass vs. infectivity), and type of animal model prevent a direct comparison with our results.

As NDV is a respiratory virus, in our vaccine trial we opted to deliver NDV intranasally, as already successfully attempted in other studies (Bukreyev et al., 2005; DiNapoli et al., 2007, 2010). Activation of the local mucosa-associated lymphoid tissue has the potential to markedly decrease nasal shedding of the virus (Gallo et al., 2021), which is of paramount importance to curtail circulation of SARS-CoV-2 in a partially immune population, and therefore limit the development of vaccine escape variants. Notably, in mice, complete protection against SARS-CoV-1 challenge is afforded only upon intranasal, but not subcutaneous, vaccine administration

(Zhao et al., 2016). Although in our study we did not test development of SARS-CoV-2-specific mucosal immunity due to lack of reagents, in the prime-boost schedule NDV-FLS-treated hamsters had no detectable infectious SARS-CoV-2 in the turbinates or the upper and lower lungs day 5 pi, suggesting development of sterilizing immunity. However, considering that low-level shedding of infectious virus was observed at day 2 pi even in vaccinated groups, vaccination may not protect against the early phases of SARS-CoV-2 replication. It is unclear if such low amounts of infectious virus would be able to infect other animals, or even if the detected virus may – at least in part – represent left-over inoculum. Lastly, as the prime-boost group in our study showed a clear increase of S-specific antibodies after the second dose, it is likely that immunity against the vector backbone did not impact the efficacy of a booster shot.

Many COVID-19 vaccine candidates are in various stages of clinical development, with some currently being given emergency-use approval in several countries. However, due to the relative advantages and disadvantages of different vaccine platforms, there is an ongoing need to develop and test novel vaccine platforms and strategies. This will also be critical in the case of potential future pandemics and emerging and re-emerging infections, which will require swift development of vaccine candidates. The prospect of having several different platforms available for rapid development should a novel pathogen arise is of critical importance. Live viral vectors are particularly advantageous due to their generally high immunogenicity, ability to induce both humoral and cellular immune responses, and the lack of a need for adjuvants (Vrba et al., 2020). This work provides evidence that this platform can provide substantial protection against SARS-CoV-2 infection and could be a viable option for further clinical development.

Limitations of the study

Oral and rectal swabs were taken on day two post-challenge with SARS-CoV-2 primarily to confirm viral replication in our hamster infection model; therefore, we did not take swabs on day five post-challenge. As these swabs were taken at such an acute time point, we cannot know for certain whether the virus or RNA detected was from leftover inoculum, at least in the oral swabs. Additionally, although the vaccine was administered to hamsters intranasally, the method of administration used would have resulted in vaccine reaching the distal lungs, where it could potentially replicate. To evaluate the true potential of intranasal administration of an NDV-based COVID-19 vaccine, a larger animal model such as a sheep, in which the efficacy of intranasal administration could be evaluated separate from pulmonary administration, would be required. Indeed, large animal studies to address efficacy of intranasal vs. pulmonary administration are currently ongoing.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

Conceptualization, S.K.W., L.S., B.W.B, P.P.M., D.K., B.M.W., and B.D.G.; methodology, S.K.W., L.S., B.W.B., B.D.G., B.M.W., and D.K.; animal experiments (including vaccination, challenge, virus titration, and serology), B.M.W, M.C., R.V., N.T., E.V., A.L., S.H., B.D.G., J.A., M.H., K.T., A.A., K.L.F., and D.K., R.C.M, LC, Y.M., J.P.K., and J.A.M.; virus engineering and purification, L.A.S., Y.P., and J.G.E.Y.; vaccine characterization, A.L., P.H.P., and J.G.E.Y; microscopic pathology assessment, L.S; writing – original draft, B.M.W., S.K.W., L.S., and D.K.; funding acquisition and resources, B.W.B., S.K.W., L.S., and D.K.; supervision, S.K.W., L.S., B.W.B, D.K., L.B., D.S., H.W., and S.B.

DECLARATION OF INTERESTS

L.A.S., Y.P., B.W.B., P.P.M., L.S., and S.K.W. are co-inventors on a United States Provisional Application No. 63/196,489 entitled "ENGINEERED NEWCASTLE DISEASE VIRUS VECTOR AND USES THEREOF", which was filed June 3, 2021.

INCLUSION AND DIVERSITY

We worked to ensure sex balance in the selection of non-human subjects. One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in science. One or more of the authors of this paper self-identifies as a member of the LGBTQ + community. One or more of the authors of this paper self-identifies as living with a disability. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-NDV ribonucleoprotein	Novus Biologicals	NBP2-11633
Goat anti-Mouse IgG conjugated to Alexa Fluor 488	ThermoFisher	Cat# A-11001; RRID:AB_2534069
Rabbit anti-SARS-CoV-2 nucleocapsid	ThermoFisher	Cat# PA5-81794; RRID:AB_2788968
Rabbit anti-SARS-CoV-2 S1	ThermoFisher	Cat# PA5-81795; RRID:AB_2788969
Rabbit anti-SARS-CoV-2 S2	Novus Biologicals	Cat# NB100-56578; RRID:AB_838846
Mouse anti-beta actin	ThermoFisher	Cat# MA5-15739; RRID:AB_10979409
Goat anti-rabbit IgG conjugated to horseradish peroxidase	ThermoFisher	Cat# G-21234; RRID:AB_2536530
Goat anti-mouse IgG conjugated to horseradish peroxidase	ThermoFisher	Cat# G-21040; RRID:AB_2536527
Goat anti-hamster IgG (H+L) conjugated to horseradish peroxidase	KPL	5220-0371
Bacterial and virus strains		
NEB® Stable Competent E. coli (High Efficiency)	New England Biolabs	C3040I
SARS-CoV-2; hCoV-19/Canada/ON-VIDO-01/2020, GISAID accession# EPI_ISL_425177	Sunnybrook Research Institute	NA
NDV-FLS	University of Guelph	NA
NDV-Δ19S	University of Guelph	NA
NDV-GFP	University of Guelph	NA
Chemicals, peptides, and recombinant proteins		
2.5% Trypsin (10X)	ThermoFisher	15090-046
Protease inhibitor cocktail	ThermoFisher	0087785
Pierce SuperSignal West Pico PLUS Chemiluminescent Substrate	ThermoFisher	34580
L-poly-L-lysine	Millipore Sigma	P4707
Iodixanol (OptiPrep™ Density Gradient Medium)	Millipore Sigma	D1556
Lactose	Millipore Sigma	17814
Peptone	Millipore Sigma	P6838
SARS-CoV-2 (2019-n-CoV) Spike protein (S1+S2 ECD, His Tag)	Sino Biological	40589-V08B1
SARS-CoV-2 (2019-nCoV) Nucleocapsid-His recombinant Protein	Sino Biological	40588-V08B
1 Step Ultra TMB-ELISA substrate	ThermoFisher	34028
Carboxymethylcellulose	Millipore Sigma	C5678
Crystal Violet, 0.5% Solution	Fisher	S25275B
10% neutral-buffered formalin	Fisher	22-220682
Critical commercial assays		
Pierce BCA Protein Assay Kit	ThermoFisher	23225
Pierce Firefly luciferase Glow Assay Kit	ThermoFisher	16176
QIAamp Viral RNA mini kit	Qiagen	52906
RLT Buffer	Qiagen	79216
RNeasy Plus Mini kit	Qiagen	74134
TaqPath 1-Step Multiplex Master Mix kit	ThermoFisher	A28525
Experimental models: Cell lines		
Vero	ATCC	CCL-81
Vero E6	ATCC	CRL-1586

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
DF-1	ATCC	CRL-12203
HEK293-hACE2	Dr. Paul Spagnuolo, University of Guelph	NA
Specific pathogen-free eggs	Canadian Food Inspection Agency	NA
Experimental models: Organisms/strains		
Syrian hamsters	Charles River	Strain code 049
Oligonucleotides		
E_Sarbeco_F1 (′- ACAGGTACGTTAATAGTTAATAGCGT-3′)	WHO	NA
E_Sarbeco_R2 (5′-ATATTGCAGCAGTA CGCACACA-3′)	WHO	NA
probe E_Sarbeco_P1 (5′-FAM-ACACTAG CCATCCTTACTGCGCTTCG -BBQ-3′)	WHO	NA
SARS-CoV-2 Spike, forward 5′GCACCGAGTTCCCCCTCTAGATTAGAAAAAAT ACGGGTAGAACCGC CAC-3′	IDT	NA
SARS-CoV-2 Spike, reverse 5′GTTGGACCTTGG GTACGCGTTTATCAGGTGT AGTGCAGCTTCAC-3′	IDT	NA
SARS-CoV-2 Spike (SΔ19), reverse 5′GTTGGACCTTGGGTACGCGTTTATCAT CAGCA GCAAGAGCCGCAAGAACAAC-3′	IDT	NA
Software and algorithms		
PRISM	GraphPad software	Version 8
BioRad Image Lab 6.0.1. software	BioRad	Version 6.0.1
Other		
Polyvinylidene difluoride (PVDF) membrane	Cytiva	10600029

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Sarah Wootton, University of Guelph (kwootton@uoguelph.ca).

Materials availability

Plasmids generated in this study are available upon request following execution of a material transfer agreement (MTA).

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics statement

The animal experiments described were carried out at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada. Studies were approved by the Animal Care Committee at the Canadian Science Center for Human and Animal Health in accordance with the guidelines provided by the Canadian Council on Animal Care. All procedures including vaccinations, infections, swabs, collections were

performed under anesthesia, and all efforts were made to minimize animal suffering and to reduce the number of animals used. All SARS-CoV-2 infectious work was performed under containment level 4 (CL-4) conditions. Equal numbers of male and female hamsters four to six weeks of age were monitored daily for any adverse signs following vaccinations and infections, and were provided food and water *ad libitum*.

Cells

Vero (ATCC CCL-81) and Vero E6 (CRL-1586) cells were maintained in Minimum Essential Media (MEM) (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich). DF-1 cells (ATCC CRL-12203) were maintained in DMEM supplemented with 10% bovine calf serum (BCS). HEK293-hACE2 cells grown on tissue culture plates coated with L-poly-L-lysine and maintained in DMEM supplemented with 10% FBS.

METHOD DETAILS

Engineering and rescue of recombinant NDV vaccines

The full-length cDNA genome of lentogenic NDV LaSota strain was designed based on Genbank accession AF077761.1 to contain a GFP reporter gene and essential NDV-specific RNA transcriptional signals, flanked by a 5′ XbaI site and a 3′ MluI site at nucleotide position 3143 between the P and M genes. A leucine-to-alanine mutation at position 289 was also introduced into the fusion gene. The full-length recombinant clone was synthesized *de novo* using a synthesis service (GeneArt, ThermoFisher). To construct recombinant NDV expressing SARS-CoV-2 Spike, forward 5′GCACCGAGTTCCCCCTCTAGATTAGAAAAAA TACGGGTAGAACCGC CAC-3′ and reverse 5′GTTGGACCTTGGGTACGCGTTTATCAGGTGTAGTG CAGCTTCAC-3′ primers were used to amplify human codon optimized SARS-CoV-2 full-length spike. Additionally, a 19 amino acid-truncated form of the S protein (SΔ19) was amplified using the previous forward primer and a reverse 5′GTTGGACCTTGGGTACGCGTTTATCATCAGCA GCAAGAGCCGCAAGAA CAAC-3′. Infusion Cloning™ was used to insert transgenes into the NDV backbone according to the manufacturer's protocol (Takara Bio USA), with the 5′ end of the primer spanning 15 bp of homology with each end of the linearized vector including the XbaI or MluI sites. Viruses were rescued from cDNA as previously described (Santry et al., 2018), and recombinant virus identity confirmed by RT-PCR and sequencing.

Challenge virus and vaccine candidates

The SARS-CoV-2 used in this study (SARS-CoV-2; hCoV-19/Canada/ON-VIDO-01/2020, GISAID accession# EPI_ISL_425177) was isolated from a clinical specimen at Sunnybrook Research Institute (SRI)/ University of Toronto on VeroE6 cells and provided to us by the Vaccine and Infectious Disease Organization (VIDO) with permission. The virus was grown in Vero cells (ATCC, CCL-81) in minimum essential medium (Hyclone) containing 1% fetal bovine serum (FBS) and 1% L-glutamine, and a passage 2 (P2) virus stock was used for all infections. The virus was titrated on Vero cells by conventional limiting dilution assays and reported as $TCID_{50}$, as described previously (Griffin et al., 2020).

Rescued NDV-FLS, NDV-Δ19S, and NDV-GFP were grown in specific pathogen-free (SPF) eggs (Canadian Food Inspection Agency), harvested from allantoic fluid, and stocks were purified according to previously published methods (Santry et al., 2018). Recombinant NDVs were titrated in DF-1 cells by limiting dilution coupled with immunofluorescent staining (see below).

Immunofluorescence assay and titration of recombinant NDV vaccines

NDV vaccine stocks were titered on DF-1 cells (immortalized chicken embryo fibroblasts [ATCC CRL-12203]) by immunofluorescence (IFA) assay. Cells were plated in 96-well plates ($4x10^4$ cells / well) in DMEM supplemented with 2% bovine calf serum (BCS) and 5% allantoic fluid, left to adhere overnight, and infected the next day with serial 10-fold dilutions of purified allantoic fluid containing recombinant NDV vaccine. Approximately 24 hr post-infection, cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature (RT), and permeabilized in 0.1% NP-40 for 10 minutes at RT. Antigens were masked in blocking buffer [5% (v/v) normal goat serum in PBS-T] either for one hour at RT or overnight at 4°C, followed by incubation with a primary mouse anti-NDV ribonucleoprotein (NBP2-11633, monoclonal; Novus Biologicals) diluted 1:2000 in blocking buffer (one hour at RT or overnight at 4°C). The secondary antibody was a goat-anti-mouse conjugated with Alexa Fluor 488 (A-11001, ThermoFisher) diluted 1:1000, which was applied in PBS-T for one hour at RT in the dark. Cells were imaged using an Axio Observer inverted

fluorescent microscope. For titration, positive wells per dilution were tallied and titer reported as $TCID_{50}$/mL, according to the Spearman-Karber method (Karber, 1931).

Vaccine characterization in DF-1 cells

DF-1 cells were seeded into 6-well plates at $1.5x10^6$ cells/well in 1 mL of DMEM with 2% bovine calf serum and supplemented with 5% allantoic fluid or 100 μg/ml of trypsin (ThermoFisher), to provide proteolytic activity. After adherence, cells were infected with either NDV-FLS, NDV-Δ19S or NDV-GFP at a multiplicity of infection (MOI) of 1 or 10 in replicate plates and incubated at 37°C. Approximately 24 hours after infection, plates were observed under an inverted phase contrast microscope to examine and document cytopathic effect. Subsequently, one set of replicate plates was used for IFA as described above, and another was used for protein extraction and western blot analysis (see below).

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot analysis

DF-1 cells were infected in 6-well plates as described above (MOI = 1), washed with PBS and lysed for 30 min in radioimmunoprecipitation assay buffer [50 mM Tris-HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1X protease inhibitor cocktail (0087785, ThermoFisher)]. Cell lysates were centrifuged at 10,000 ×g for 15 min at 4°C, supernatants collected and used to quantify the protein concentration using the Pierce BCA Protein Assay Kit (ThermoFisher). For SDS-PAGE, purified virus ($1x10^7$ PFU) or virus infected cell lysates (mixed with 6x loading dye containing and 30% β-mercaptoethanol) were heated at 95°C for 10 min to denature proteins, followed by cooling on ice. The same PFU or protein amounts of each sample (ranging from 5 to 70 μg depending on the experiment) were loaded into wells of 4% stacking / 12% resolving gels, and proteins were resolved at 120 V for 1.5 h in running buffer (0.025 mM Tris-base, 0.192 M glycine, 0.1% SDS). Proteins were transferred to a 0.2 μm polyvinylidene fluoride or polyvinylidene difluoride (PVDF) membrane for 30 min, using the BioRad Trans-Blot Turbo Transfer System and associated buffer (BioRad Trans-Blot Turbo RTA Mini PVDF Transfer Kit). Following transfer, the rest of the protocol was performed as previously described (Pham et al., 2020). Briefly, the primary antibodies were incubated overnight at 4°C, and were either mouse anti-NDV ribonucleoprotein (dilution: 1:5000; NBP2-11633; Novus Biologicals), rabbit anti-SARS-CoV-2 nucleocapsid (dilution: 1:5000; PA5-81794; ThermoFisher), rabbit anti-SARS-CoV-2 S1 (dilution: 1:1000; PA5-81795; ThermoFisher) or S2 (dilution: 1:1000; NB100-56578; Novus Biologicals) subunits, or mouse anti-beta actin (diluted 1:1000; MA5-15739; ThermoFisher). The secondary antibodies were either goat anti-rabbit (G-21234) or goat anti-mouse IgG (G-21040) conjugated to horseradish peroxidase (diluted 1:2000; ThermoFisher), and incubated for 1 to 3 h at RT. Protein was detected using the Pierce SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher) and the BioRad ChemiDoc MP Imaging System (BioRad Image Lab 6.0.1. software).

Neutralization of pseudotyped lentiviruses and NDV recombinant vaccines

To evaluate the contribution of the S protein to the infectivity of NDV-FLS and NDV- Δ19S, neutralization assays were conducted using immune chicken serum against NDV and murine serum raised against the S protein (see below). The chicken serum was collected from routinely vaccinated White Leghorn hens (Arkell Research Station, University of Guelph).

Briefly, mice were vaccinated intramuscularly with 10^8 infectious units (IU) of adenovirus expressing FLS, in an homologous prime-boost regimen 28 days apart. The neutralizing activity of the serum was tested on lentiviral particles pseudotyped with the truncated version of the S protein (Δ19S) and encoding the luciferase gene. Neutralization was carried out in a 96-well plate format. Approximately 10,000 HEK cells expressing the human ACE-2 receptor (HEK-ACE2) were plated in each well using L-poly-L-lysine (Millipore Sigma) to improve adherence. Equal amounts of pseudotyped lentiviruses were added to the wells (approximately $8x10^8$ relative light units [RLU] / well) and incubated with two-fold dilutions of mouse serum starting from a 1:100 dilution. Luminescence intensity was evaluated after three days using the Pierce Firefly luciferase Glow Assay Kit (ThermoFisher), and RLUs quantified using an EnSpire Multimode Plate Reader (PerkinElmer). At 1:100 dilution, the serum of vaccinated mice showed to decrease the RLU of approximately 80-90%, compared to wells incubated with the serum of non-vaccinated mice or no serum controls (Figure S2).

Neutralization of NDV-FLS and NDV- Δ19S was done in 96 well plates, separately for mouse and chicken sera. Poly-L-lysin-coated wells were seeded with HEK-ACE-2 cells and let adhere overnight. Equal amounts of NDV-FLS or NDV- Δ19S (1,000 PFU in 50 μl) were added to each well containing 1:2 dilutions of mouse or chicken serum in 50 μl, starting from an initial 1:100 dilution. Total volume for each well was 100 μl / well, and the final solution included 5% allantoic fluid. After 3 days, the magnitude of infection was evaluated by IFA for NDV ribonucleoprotein, as described above. The amount of IFA signal in the wells tested with murine serum was quantified by image analysis (ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA), by averaging the signal intensity of the fluorescent signal using pictures from 4 different wells.

Determination of mean death time (MDT)

The MDT was determined for the two vaccine candidates and the control (NDV-FLS, NDV-SΔ19, and NDV-GFP). The virus stocks were equalized to a starting titer of 6.14×10^6 $TCID_{50}$/mL, and each virus stock was serially diluted from 10^{-1} to 10^{-8} in PBS. Dilutions from 10^{-4} to 10^{-8} were inoculated into specific pathogen-free eggs (Canadian Food Inspection Agency) at 9 to 11 days of embryonation. For each virus, five eggs were inoculated per dilution (100 μL / egg). The experiment was run in duplicate 3 to 4 hours apart, resulting in 50 eggs used for each virus. After virus inoculation, the eggs were incubated for up to 7 days and checked twice daily for embryo mortality; after the first 24 hr after inoculation, allantoic fluid was collected from all dead embryos to check for presence of NDV by hemagglutination assay (HA), according to standards methods (McGinnes et al., 2006). The MDT was recorded as the time (in hours) taken by the minimal lethal dose (highest dilution) to kill all five eggs in the dilution series. If no minimal lethal dose was observed by the end of the experiment (seven days post-inoculation), it was concluded that the MDT was > 168 hrs, and HA was performed on the allantoic fluid collected from eggs inoculated with the lowest virus dilution (10^{-4}) to confirm NDV infection.

Lyophilization of NDV-FLS

Triplicate samples of freshly harvested allantoic fluid containing NDV-FLS were aliquoted into 15mL conical tubes in 1mL volumes. Aliquots were either left untreated or adjusted to a final concentration of 5% sucrose, 5% sucrose/5% Iodixanol or mixed 1:1 with a solution containing 10% lactose, 2% peptone, 10mM Tris-HCl, pH 7.6. Using an LABCONCO Freeze Dry system Freezone®4.5, samples were immediately lyophilized at 44 x 10-3 MBAR and -52°C for 16 hours. Lyophilized samples were stored at 4°C for 48 hours before being resuspended in 1 mL of 5% sucrose/PBS and titered. Three 1 mL aliquots of allantoic fluid containing NDV-FLS were adjusted to 5% sucrose and frozen at -70°C before titering. An additional three 1 mL aliquots were used to titer NDV-FLS in allantoic fluid immediately following harvest from eggs. All samples were titered by $TCID_{50}$ on DF-1 cells as described above.

To determine presence of S protein in the reconstituted preparations, lyophilized (10% lactose and 2% peptone group, only) and reconstituted NDV-FLS was compared to NDV-FLS stored at -70°C (purified preparation frozen at -70°C in sucrose). Equal amounts of virus preparations (10^7 PFU, as determined by post-reconstitution titers) were loaded on SDS-PAGE gel, transferred to a nitrocellulose membrane and immunoblotted for the S protein using a rabbit anti-SARS-CoV-2 S2 subunit (dilution: 1:1000; NB100-56578; Novus Biologicals), as described in the previous section. To determine the ability of lyophilized and reconstituted virus to express S protein in infected cells, DF-1 cells were infected with the same amounts of reconstituted or frozen virus (MOI = 0.5). Whole cell lysates were harvested 24 hrs post-infection, immunoblotting for the S protein was conducted as above.

Immunization and infection of Syrian hamsters

For initial immunization and booster immunization of hamsters, groups of ten Syrian Golden hamsters (five male and five female, four to six weeks of age; Charles River) were anaesthetized with inhalation isoflurane and administered 1×10^7 PFU of recombinant NDV-GFP, NDV-FLS, or NDV-Δ19S via the intranasal (IN) route. For IN vaccinations, anaesthetized hamsters were scruffed and vaccines were delivered in a 100 μL volume (q.s. with PBS) through the nares (50 μL per nare). Animals had their mouths held closed to ensure inhalation through the nose. After recovery from anesthesia hamsters were monitored daily for any adverse signs following vaccine administration.

For SARS-CoV-2 infection following immunization, hamsters were moved into a CL-4 facility and then anaesthetized with inhaled isoflurane. Hamsters were then infected with 10^5 $TCID_{50}$ of SARS-CoV-2 via

the same IN method described above. After recovery from anesthetic hamsters were monitored daily throughout the course of infection. Body weights and temperatures of hamsters were recorded daily.

Microscopic pathology

At day five post-challenge, six hamsters per group were euthanized, and the proximal and distal lobes of the lung from each hamster were sampled and fixed in 10% buffered formalin, followed by routine paraffin embedding, sectioning, and staining with hematoxylin and eosin (HE). The magnitude of microscopic lesions caused by SARS-CoV-2 in the lungs of vaccinated and control mice was evaluated histologically using two semi-quantitative scoring systems based on the presence of nominal categories (Tables S1 and S2) (Meyerholz and Beck, 2020) and extent of the pulmonary parenchyma affected (Table S3) (Imai et al., 2020). Assessment was carried out taking into consideration all the sections available for evaluation. Slides were scored by a board-certified veterinary anatomic pathologist (LS), who was blind to the treatment of the experimental groups (group de-identification).

Detection of SARS-CoV-2 RNA in tissues and swabs of infected hamsters

Oropharyngeal and rectal swabs were obtained and stored in MEM + 2% penicillin-streptomycin. For viral RNA detection, 140μL of the medium containing the swab was used for viral lysis and extraction using the QIAamp Viral RNA mini kit. For viral RNA detection, tissue samples were thawed, weighed, and then homogenized in 600 μL RLT buffer (Qiagen) using a Bead Ruptor Elite Bead Mill Homogenizer (Omni International) with a stainless steel bead for at 4 m/s for 60 seconds. Viral RNA from 30 mg samples of each tissue was extracted with the RNeasy Plus Mini kit (Qiagen) according to manufacturer's instructions, and viral RNA from swab samples was extracted with the QIAamp Viral RNA Mini kit (Qiagen) also according to manufacturer's instructions. Detection of SARS-CoV-2 E gene was performed using TaqPath 1-Step Multiplex Master Mix kit (Applied Biosystems) and was carried out on a QuantStudio 5 real-time PCR system (Appiled Biosystems), as per the manufacturer's instructions. RNA was reverse transcribed and amplified using the primers reported by the WHO and include E_Sarbeco_F1 (5′- ACAGGTACGTTAATAGTTAATAGCGT-3′) and E_Sarbeco_R2 (5′-ATATTGCAGCAGTA CGCACACA-3′) and probe E_Sarbeco_P1 (5′-FAM-ACACTA GCCATCCTTACTGCGCTTCG -BBQ-3′). A standard curve produced with synthesized target DNA was run with every plate and used for the interpolation of viral genome copy numbers.

Detection of infectious SARS-CoV-2 in tissues and swabs of infected hamsters

For infectious virus assays, thawed tissue samples were weighed and placed in 1 mL of minimum essential medium supplemented with 1% heat-inactivated fetal bovine serum (FBS) and 1x L-glutamine, then homogenized in a Bead Ruptor Elite Bead Mill Homogenizer (Omni International) at 4 m/s for 30 seconds then clarified by centrifugation at 1,500 xg for 10 minutes. Prior to titration procedures, oropharyngeal and rectal swabs stored in MEM + 2% penicillin-streptomycin were vortexed and centrifuged briefly. Samples were serially diluted 10-fold in media and dilutions were then added to 96-well plates of 95% confluent Vero cells containing 50 μL of the same medium in replicates of three and incubated for five days at 37°C with 5% CO_2. Plates were scored for the presence of cytopathic effect on day five after infection. Titers were calculated using the Reed-Muench method, and reported as $TCID_{50}$ units.

Determination of antibody responses by ELISA and PRNT assay

For detection of anti-SARS-CoV-2-specific antibody responses, all hamsters were bled via jugular vein bleeds for serum on days 21, 29, 49, and 56 post-first vaccination. For ELISAs for detection of total IgG detection, SARS-CoV-2 spike- and nucleoprotein-specific responses were assessed using an in-house assay. A 1:400 dilution of serum was carried out in duplicate and added to plates pre-coated with both spike and nucleoprotein in the same assay wells. IgG was detected with a peroxidase-labeled polyclonal goat anti-hamster IgG (H+L) (KPL).

For virus PRNT (plaque reduction neutralization assays), serum samples were heat-inactivated at 56°C for 30 minutes and diluted two-fold from 1:40 to 1:1280 in DMEM supplemented with 2% FBS. Diluted sera were incubated with 50 PFU of SARS-CoV-2 at 37°C and 5% CO2 for 1 hour. The sera-virus mixtures were added to 24-well plates containing Vero E6 cells at 100% confluence, followed by incubation at 37°C and 5% CO2 for 1 hour. After adsorption, 1.5% carboxymethylcellulose diluted in MEM supplemented with 4% FBS, L-glutamine, non-essential amino acids, and sodium bicarbonate was added to each well and plates were incubated at 37°C and 5% CO2 for 72 hours. The liquid overlay was removed and cells were

fixed with 10% neutral-buffered formalin for 1 hour at room temperature. The monolayers were stained with 0.5% crystal violet for 10 minutes and washed with 20% ethanol. Plaques were enumerated and compared to a 90% neutralization control. The PRNT-90 endpoint titer was defined as the highest serum dilution resulting in a 90% reduction in the number of plaques. PRNT-90 titers $\geq$1:40 were considered positive for neutralizing antibodies.

Hematological and biochemical analysis of blood and serum

Complete blood counts were carried out using a VetScan HM5 hematology system (Abaxis Veterinary Diagnostics), as per the manufacturer's instructions. Analysis of serum biochemistry was performed with a VetScan VS2 analyzer (Abaxis Veterinary Diagnostics), as per the manufacturer's instructions.

Cytokine mRNA analysis

RNA was extracted from proximal lung samples as described above using a RNeasy Plus Mini Kit (Qiagen), which includes a genomic DNA elimination step, as per the manufacturer's instructions. Expression of IFNγ, IL-2, IL-4, IL-10, IL-1b, FoxP3, TGF-β, VEGF, IL-6, and TNFα mRNA was analyzed using the one-step TaqPath Master Mix kit using the primer/probe sets described previously (Warner et al., 2017). Ribosomal protein L18 (RPL18) was used as an internal control. All RT-qPCR assays were performed on a QuantStudio 5 instrument (Applied Biosystems). Expression is reported as Log_2 of the fold-change for each gene as calculated using the ΔΔCt method compared with expression of the same genes in sex-matched control tissues that were unvaccinated and uninfected.

QUANTIFICATION AND STATISTICAL ANALYSIS

Mean scores between experimental groups were compared using analysis of variance (ANOVA) with Tukey's post-hoc (lyophilization experiment) or Holm Sidak (neutralization) tests for multiple comparisons, two-way ANOVA (serology), non-parametric Mann-Whitney or Kruskal-Wallis test followed by the Dunn's method for multiple comparisons (all other tests and pathology data), with significance set at $p < 0.05$ as implemented in GraphPad software version 8.0.0 (San Diego, California, USA, www.graphpad.com). Data are represented as scatterplots with median, median with range, or average with standard deviation (see figure legends).

This is Exhibit Z referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

ENGINEERED NEWCASTLE DISEASE VIRUS VECTOR AND USES THEREOF

Jun 3, 2022

An engineered Newcastle Disease Virus (NDV) vector is provided. In particular, the present disclosure provides methods of treating or preventing a disease such as cancer, or an infectious disease, or methods for eliciting an immune response, with the engineered NDV vector. The engineered NDV vector provided herein is useful as an immunogenic composition, an oncolytic agent, or a vaccine.

Skip to: Description · Claims · Patent History · Patent History

Description

RELATED APPLICATION

This disclosure claims benefit and priority of U.S. Provisional Patent Application Ser. No. 63/196,489 filed Jun. 3, 2021, incorporated herein by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

A computer readable form of the Sequence Listing "P62990US01_Sequence_Listing_ST25" (426,627 bytes), submitted via EFS-WEB and created on Jun. 3, 2022, is herein incorporated by reference.

FIELD

The present disclosure provides engineered Newcastle Disease Virus (NDV) vectors comprising a nucleic acid having a nucleic acid sequence described herein. The NDV vectors may comprise at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a viral promoter capable of expressing the segment in a host cell. Also provided are methods of treating a disease with said engineered NDV vectors and a vaccine comprising an engineered NDV vector described herein, and methods of treating a disease with said vaccine.

BACKGROUND

Newcastle Disease Virus (NDV), also known as avian orthoavulavirus-1 (AOaV-1), is an enveloped avian paramyxovirus virus with a non-segmented, negative-sense RNA genome. NDV has been studied as a candidate engineered live vaccine platform for human and veterinary infectious diseases. NDV may be useful as a candidate vaccine vector for a few reasons. As an avian virus, NDV is antigenically distinct from common human vaccines and pathogens, averting the problem of pre-existing immunity that would limit its efficacy in people. As an oncolytic agent, NDV has shown an excellent safety profile, whereby direct intravenous, aerosol, or intratumoral administration of large virus doses is well tolerated in people (Wheelock, E. F. and J. H. Dingle, 1964; Csatary, L. K., et al., 1993; Pecora, A. L, 2002). As a vaccine vector in pre-clinical models, NDV-vectored vaccines have been shown to be safe and protective in non-human primate models of pathogenic avian influenza,

Ebola, and SARS-CoV-1 (severe acute respiratory syndrome coronavirus-1) (Bukreyev, A., et al., 2005; DiNapoli, J. M., et al., 2010; DiNapoli, J. M., et al., 2007). Additionally, the NDV viral genome is highly versatile, allowing for stable insertion and high-level expression of foreign genes such as viral antigens. Lastly, NDV is an acute cytoplasmic virus and its genomic RNA is tightly encapsidated by nucleocapsid protein; all features that markedly mitigate concerns about insertional mutagenesis or recombination.

The novel SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) emerged in late 2019 as the causative agent of a severe respiratory disease named coronavirus disease 2019 (COVID-19). The virus has been classified in the Coronaviridae family, β-coronavirus genus, and Sarbecovirus subgenus (i.e., β-coronavirus subgroup B). Phylogenetic analysis has shown that this virus shares ≈50% genetic similarity with MERS (Middle East Respiratory Syndrome)-CoV, ≈with SARS-CoV-1, and >90% similarity with bat β-coronaviruses. SARS-CoV-2 is transmitted through contact and respiratory route. In people with severe disease, morbidity and mortality are mediated by severe respiratory distress syndrome and vascular disease. The former is caused by diffuse alveolar damage associated with virus replication in type I and II alveolar pneumocytes. Molecular effectors of tissue damage include unchecked production of pro-inflammatory cytokines (i.e., cytokine storm), decreased angiotensin-converting enzyme-2 (ACE2) activity, and activation of a thrombo-inflammatory cascade leading to a hypercoagulable state.

Multiple research groups have been working towards production of several vaccine platforms against SARS-CoV-2, including engineered viral vectors, nucleic acids (DNA, mRNA and self-replicating RNA), protein subunits, virus-like particles, and live-attenuated or inactivated SARS-CoV-2 virions. The vast majority of these vaccines target the SARS-CoV-2 Spike (S) protein, the main neutralizing antigen against the virus. In December 2020, two mRNA based COVID-19 vaccines (Pfizer-BioNTech and Moderna) received emergency use authorization by the U.S. Food and Drug Administration; however, it is unclear whether these vaccines will have reduced efficacy against Variants of Concern (VoC), such as the South African B.1.351 variant, highlighting the need for vaccines that induce sterilizing immunity (Peiris, M. and G. M. Leung, 2020).

Due to the relative advantages and disadvantages of different vaccine types, there is an ongoing need to develop and test novel vaccine platforms and strategies. New vaccines may be critical for potential future pandemics and emerging and re-emerging infections, which will require swift development of vaccine candidates. Live viral vectors may be useful due to their generally high immunogenicity, ability to induce both humoral and cellular immune responses, and the lack of a need for adjuvants.

SUMMARY

The present inventors produced an engineered (fully synthetic) Newcastle Disease Virus (NDV) vector, which is immune stimulatory and useful as a therapeutic agent for oncolytic viral therapy, or as a vaccine platform for immunoprophylaxis. In particular, the inventors created an intra-nasally delivered, non-virulent NDV vaccine expressing the SARS-CoV-2 spike protein for protecting subjects from COVID-19 or related coronaviruses. The use of a non-virulent NDV strain (i.e., lentogenic pathotype) makes the vaccine safe in both mammals and avian species, including poultry, which are the natural target of NDV. Intra-nasal delivery stimulates both a mucosal and systemic immune response in the host, and a needle-free administration is logistically simpler and can ameliorate concerns associated with vaccine hesitancy. The engineered NDV vector of this disclosure can infect host cells to express an immunogenic agent, for example, the SARS-CoV-2 spike protein (NDV-FLS), which leads to the production of spike protein-specific serum IgG and mucosal IgA antibodies as well as spike protein-specific T cells responses in subjects administered the vaccine intranasally.

Accordingly, the present disclosure provides an engineered Newcastle Disease Virus (NDV) vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequences encoding phosphoprotein and matrix protein.

The present disclosure also provides a method of treating or preventing a disease in a subject, comprising administering an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

Also provided is use of an engineered NDV vector for treating or preventing a disease in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

Further provided is use of an engineered NDV vector in the manufacture of a medicament for treating or preventing a disease in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

Even further provided is an engineered NDV vector for use in treating or preventing a disease in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

In an embodiment, the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27. In an embodiment, the engineered NDV vector comprises a nucleic acid having a nucleic acid

sequence that is at least 95% identical to the nucleic acid sequence any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42. In an embodiment, the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 99% identical to the nucleic acid sequence any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42. In an embodiment, the engineered NDV vector comprises a nucleic acid sequence consisting of the nucleic acid sequence any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42.

In an embodiment, the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus. In an embodiment, the stabilizing segment comprises an amino acid sequence as set forth in SEQ ID NO: 20. In an embodiment, the stabilizing segment is encoded by a nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO: 35. In an embodiment, the chimeric F protein comprises at the C-terminus 53 amino acid of NDV F protein from amino acid positions 501 to 553 of SEQ ID NO: 28. In an embodiment, the chimeric HN protein comprises at the N-terminus 53 amino acids of NDV HN protein from amino acid positions 1 to 53 of SEQ ID NO: 34. In an embodiment, the engineered NDV vector of any one of claims **8** to **11**, wherein the L protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence as set forth in SEQ ID NO: 11. In an embodiment, the chimeric F protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 12. In an embodiment, the chimeric HN protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 13.

In an embodiment, the NDV vector is lentogenic, and wherein the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 25.

Also provided is an engineered Newcastle Disease Virus (NDV) vector comprising a nucleic acid having a nucleic acid sequence encoding an L protein having a stabilizing segment, a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus. In an embodiment, the nucleic acid comprises XbaI and MluI restriction

endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the stabilizing segment comprises an amino acid sequence as set forth in SEQ ID NO: 20. In an embodiment, the stabilizing segment is encoded by a nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO: 35. In an embodiment, the chimeric F protein comprises at the C-terminus 53 amino acid of NDV F protein from amino acid positions 501 to 553 of SEQ ID NO: 28. In an embodiment, the chimeric HN protein comprises at the N-terminus 53 amino acids of NDV HN protein from amino acid positions 1 to 53 of SEQ ID NO: 34. In an embodiment, the L protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence as set forth in SEQ ID NO: 11. In an embodiment, the chimeric F protein comprises an amino acid sequence having at least 85% identity to the amino acid sequence of SEQ ID NO: 12. In an embodiment, the chimeric HN protein comprises an amino acid sequence having at least 85% identity to the amino acid sequence of SEQ ID NO: 13. In an embodiment, the NDV vector is lentogenic, and wherein the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 25. In an embodiment, the nucleic acid further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell.

In another embodiment, the host cell is selected from the group consisting of a human, primate, murine, feline, canine, ovine, bovine, porcine, caprine, equine, lupine, vulpine, mustelid host cell and. In a further embodiment, the promoter is capable of expressing the at least one heterologous nucleic acid segment encoding the therapeutic agent in muscle, airways, or lung cells.

In an embodiment, the disease is an infectious disease. In an embodiment, the infectious disease is selected from the group consisting of viral diseases such as viral hemorrhagic fevers, Ebola, Marburg virus disease, gastroenteritis, dengue fever, West Nile fever, yellow fever, influenza, respiratory syncytial virus disease, Lassa fever, rabies, smallpox, cowpox, horsepox, monkeypox, Hantavirus pulmonary syndrome, Hendra virus disease, Nipah virus disease, human immunodeficiency virus infection and acquired immunodeficiency disease syndrome, Hepatitis, Zika fever, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), Coronavirus disease 2019 (COVID-19), infectious bronchitis, infectious laryngotracheitis, Rift Valley fever, porcine epidemic diarrhea, porcine transmissible gastroenteritis, swine acute diarrhea syndrome, feline infectious peritonitis, African swine fever, classical swine fever, and bacterial diseases including drug resistant bacterial diseases such as tuberculosis and methicillin-resistant *Staphylococcus*

aureus infection, and drug resistant parasitic diseases such as malaria. In an embodiment, the infectious disease is COVID-19.

In an embodiment, the therapeutic agent comprises a SARS-CoV-2 spike protein. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41.

In an embodiment, the subject is an animal. In an embodiment, the animal is human or a veterinary animal. In an embodiment, the subject is human. In an embodiment, the subject is a veterinary animal. In an embodiment, the veterinary animal is a primate, a murine, a feline, a canine, an ovine, a bovine, a porcine, a caprine, an equine, a lupine, a vulpine, or a mustelid. In an embodiment, the subject is a mustelid.

In another embodiment, the engineered NDV vector is administered or co-administered intravenously, intranasally, intratracheally, intramuscularly, or via aerosol. In an embodiment, the viral vector is delivered to lung cells or tissues. In an embodiment, the viral vector is delivered intranasally or intramuscularly. In an embodiment, the viral vector is delivered to an animal. In an embodiment, the viral vector is delivered to a human or a veterinary animal. In an embodiment, the veterinary animal is a primate, a murine, a feline, a canine, an ovine, a bovine, a porcine, a caprine, an equine, a lupine, a vulpine, or a mustelid. In an embodiment, the viral vector is delivered to a human. In an embodiment, the viral vector is delivered to a mustelid.

The present disclosure also provides an isolated nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the nucleic acid sequence is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27, wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell.

In an embodiment, the nucleic acid further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing

the segment in a host cell.

Further provided is a pharmaceutical composition comprising an engineered NDV vector described herein, and a pharmaceutically acceptable carrier. In an embodiment, the pharmaceutical composition is lyophilized.

Further provided is a method of producing a protein in vivo in a subject, comprising delivering or introducing into the subject an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a protein operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

Further provided is an immunogenic composition, an oncolytic agent, or a vaccine comprising an engineered NDV vector described herein for treating a disease described herein.

Further provided is a method of eliciting an immune response, comprising administering to a subject an engineered NDV vector described herein, for treating a disease described herein.

Further provided is a method of treating cancer, comprising administering to a subject an engineered NDV vector described herein, wherein the NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, 10, 23, or 27.

Further provided is a method for selecting an engineered NDV vector genome comprising a stabilizing segment in L gene, the method comprises:

- a) growing bacterial cells comprising an engineered NDV vector genome in a growth medium broth;
- b) growing the bacterial cells on an agar-growth medium, wherein the agar-growth medium comprises a selection agent;
- c) identifying small bacterial cells colonies having about 0.5 mm to about 1 mm in diameter after at least 24 hours of growth;

- d) repeating step a) to step c) two to nine times to enrich for small bacterial cell colonies; and
- e) isolating the engineered NDV vector genome from the small bacterial cells colonies,
- wherein the small bacterial cells colonies comprise stable engineered NDV vector genome having the stabilizing segment in L gene.

Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific Examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments are described below in relation to the drawings in which:

FIG. **1**A shows a schematic representation of an engineered NDV vector with XbaI and MluI restriction endonuclease sites introduced between the P and M genes. GFP, full-length spike protein (FLS) or the C-terminal truncated spike (Δ19S) genes were inserted into this site.

FIG. **1**B shows virus replication and cytopathic effect in cells. DF-1 cells were infected with NDV-FLS, NDV-Δ19S, or NDV-GFP virus at a multiplicity of infection (MOI) of 10. The first row shows immunofluorescence staining for NDV ribonucleoprotein. The second row shows bright field. Both NDV-FLS and NDV-Δ19S replicated in cells, showing accumulation of NDV nucleoprotein, and caused cytopathic effect (syncytia), similar to the NDV-GFP control.

FIG. **1**C shows results of agarose gel electrophoresis of PCR amplified products from DF-1 cells infected with engineered NDV expressing SARS-CoV-2 spike protein to confirm spike protein expression. DF-1 cells were infected with either NDV-FLS, NDV-Δ19S, or NDV-GFP. RNA was extracted from cells 12 hours later and reverse transcribed to cDNA with M-MuLV-RT. Primers were used to target both the FLS and the 119S (lanes 1-6); or only the full-length spike (lanes 7-12). Lanes 1 and 7: NDV-FLS; lanes 2 and 8: NDV-Δ19S; Lanes 3 and 9: NDV-GFP; Lanes 4 and 10: plasmid clone of NDV-full-length spike protein (positive control); Lanes 5 and 11: uninfected DF1 cells (negative control); Lanes 6 and 12: no-template control. M=GeneRuler 50 bp DNA Ladder (Thermo Fisher Scientific).

FIG. **1**D shows Western blots of whole cell lysates from DF-1 cells infected with an MOI of 5, with either (1) NDV-FLS; (2) NDV-Δ19S; (3) NDV-GFP; or (4) uninfected negative control to confirm spike protein expression. Immunoblotting was done with rabbit-anti-spike protein (NB100-56578; Novus Biologicals), mouse-anti-NDV (NBP2-11633; Novus Biologicals), and mouse-anti-actin (MA5-15739; ThermoFisher). A strong band at around 180 kDa corresponding to the spike protein is detected in the lysate of cells infected with NDV-FLS and -SΔ19, but not in cells infected with NDV-GFP or uninfected cells (control). Infection was confirmed by the presence of bands corresponding to the ribonucleoprotein for NDV in infected cells.

FIG. **1**E shows Western blots of purified viruses. 1.0×10^{7} focus forming units (FFU) of (1) NDV-FLS; (2) NDV-Δ19S; or (3) NDV-GFP vectors were used for Western blotting using a primary rabbit anti-spike protein antibody (top), or a primary mouse anti-NDV ribonucleoprotein antibody (bottom), with the same antibodies described for FIG. **1**D. The blot shows incorporation of the spike protein into the purified virions, while NDV-Δ19S and NDV-GFP control shows no transgene expression.

FIG. **1**F shows crystal violet staining of DF-1 cells infected with NDV-GFP, NDV-FLS, or NDV-Δ19S vector. DF-1 cells in 6-well plates were infected with each of NDV-GFP, NDV-FLS, or NDV-Δ19S virus at an MOI of 0.1. Cells were grown in DMEM with 2% FBS supplemented with 5% allantoic fluid. 24 hours post-infection (hpi), media was removed, cells were washed in PBS, fixed with methanol/acetone for 20 minutes at −20° C., and stained with crystal violet.

FIG. **1**G shows fusogenicity score of NDV-GFP, NDV-FLS, and NDV-Δ19S. Fusogenicity score was calculated by dividing the number of nuclei by the number of cells in four fields of view per each of the three biological replicates. Counting was assisted using ImageJ (U.S. National Institutes of Health, Bethesda, Md., USA). The score for each virus was normalized to the non-infected negative control, and averages were compared using an ANOVA and a Kruskal-Wallis multiple comparisons test. NDV-FLS showed less fusogenicity compared to the other viruses (***$p<0.001$).

FIG. **2** shows an immunoblot from cell lysates infected with NDV-FLS, NDV-Δ19S, and NDV-GFP, as well as the purified viruses. The blot shows efficient incorporation of spike protein into the NDV virion (first lane of top and middle blots, after molecular weight marker [MW]). Additionally, overexposure of a Western blot for spike protein reveals the presence of C-terminal truncated spike protein in the NDV-Δ19S virion (middle blot, rectangular box), albeit at much lower intensity than the full-length spike protein in the

NDV-FLS virion. This shows that specific cytoplasmic transport signals are needed to enable efficient incorporation of the transgene on the NDV virion's surface.

FIG. **3** shows that neutralizing antibodies directed against SARS-CoV-2 spike protein do not block NDV-FLS or NDV-Δ19S infection of HEK293T-hACE2 cells. 1000 focus-forming units (FFU) of NDV-FLS, NDV-Δ19S or NDV-GFP were incubated with an antibody against the SARS-CoV-2 spike protein receptor binding domain (MA5-35958) at multiple dilutions (10 ug/mL, 5 ug/mL, 2.5 ug/mL down to 0.31 ug/mL [1/25]) for 1 h at room temperature with rocking plus 30 min at 37° C. HEK293T-hACE2 cells (2% FBS, DMEM, 5% allantoic fluid) were infected with the virus-Ab mixture and immunofluorescence assay was performed three days post infection. Images for the first three antibody dilutions are shown. These results show that neutralizing antibodies against SARS-CoV-2 spike protein do not affect NDV-FLS or NDV-Δ19S infection. When cells were incubated with hyperimmune serum from chickens vaccinated against NDV, the NDV-FLS was fully neutralized, suggesting that additional S protein on the surface does not functionally allow the virus to enter the cells.

FIG. **4** shows lyophilized NDV-FLS virus retains infectivity. Triplicate samples of NDV-FLS were either left untreated or adjusted to a final concentration of 5% sucrose, 5% sucrose/5% Iodixanol or mixed 1:1 with a stabilizing agent comprised of 10% lactose, 2% peptone, 10 mM Tris-HCl, pH 7.6 and lyophilized at 44×10^{-3} MBAR and −52° C. for 16 hr. Lyophilized samples were stored at 4° C. for 48 hours before being resuspended in 1 mL 5% sucrose/PBS and titered by TCID50 on DF-1 cells. Statistical analysis was completed by using a two-way analysis of variance with Tukey's multiple comparisons test with significance set at $p<0.05$.

FIG. **5** shows quantification of spike protein-specific CD8+ T cell responses. Groups of male Balb/c mice were administered with 5×10^{5}, 1×10^{6} or 1×10^{6} PFU of NDV-FLS in either sucrose of iodixanol intranasally. After 32 days, mice were boosted with the same dose of vaccine via the same route (intranasal). Five days after boost, the mice were euthanized and spike protein-specific CD8 T cell responses were quantified in the blood, spleen, bronchoalveolar fluid (BALF), and lung.

FIG. **6** shows quantification of spike protein-specific CD4+ T cell responses. Groups of male Balb/c mice were administered with 5×10^{5}, 1×10^{6} or 1×10^{6} PFU of NDV-FLS in either sucrose of iodixanol intranasally. After 32 days, mice were boosted with the same dose of vaccine via the same route (intranasal). After 32 days, mice were boosted with the same dose of vaccine via the same route of administration. Five days after boost, the mice were

euthanized and spike protein-specific CD8 T cell responses were quantified in the blood, spleen, bronchoalveolar fluid (BALF), and lung.

FIG. **7** shows the kinetics of spike protein-specific CD8+ and CD4+ T cells in the blood of vaccinated mice. Male C57BL/6 or Balb/c mice were vaccinated using either intranasal or intramuscular delivery of 5×10^{6} FFU NDV-FLS, with a boost delivered through the same route and same dose 32 days post prime. At day 10 post-vaccine administration, a subset (n=4) of mice were terminally bled and the spike protein specific CD8+ and CD4+ T cell responses quantified. Mice were non-terminally bled prior to being boosted on day 28, and then bled again on days 5 and 10 post-boost. Spike protein specific CD8+ and CD4+ T cell responses were quantified in the collected blood.

FIG. **8** shows killing of murine acute myeloid leukemia (AML) C1498 cells in vitro by mesogenic NDV-GFP-GM (i.e., the mesogenic version of the NDV backbone expressing the GFP protein). C1498 cells were infected at different MOIs, spanning 0.0001 to 100, and after 72 days, the metabolic activity of infected cells was evaluated by Resazurin assay as an indication of the cytolytic potential of the tested viruses. Tested viruses include the mesogenic NDV-GFP-GM (Guelph mesogenic), the lentogenic NDV-GFP-GL, and a hyper-fusogenic mesogenic NDV-GFP-NY. Results show that NDV-GFP-GM caused a significantly higher drop in metabolic activity compared to the other two tested viruses (*$p<0.05$, **$p<0.01$, ****$p<0.0001$).

FIG. **9**A shows the percentage of NK cells expressing the early activation marker CD69, in the blood of ID8 ovarian tumor bearing mice 36 hours after intravenous injection of 1×10^{8} PFU NDV-F3aa-GFP (mesogenic). NDV: Newcastle disease virus; PBS: phosphate-buffered saline mock control group; * $p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$; ns=not significant.

FIG. **9**B shows a graph depicting the percentage of NK cells in the blood of ID8 ovarian tumor bearing mice that are IFNy+, 36 hours post intravenous injection of 1×10^{8} PFU NDV-F3aa-GFP (mesogenic). NDV: Newcastle disease virus; PBS: phosphate-buffered saline mock control group; *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$; ns=not significant.

FIG. **10** shows an immunoblot of prefusion stabilized SARS-CoV-2 spike (PFS) in the allantoic fluid of embryonated eggs inoculated with NDV-PFS. A 6% SDS-PAGE gel and rabbit anti-SARS-CoV-2 S1 (dilution: 1:1000; PA5-81795; ThermoFisher) was used for detection of SARS-CoV-2 spike (black arrow). A 10% SDS-PAGE gel and mouse anti-NDV ribonucleoprotein (dilution: 1:5000; NBP2-11633; Novus Biologicals) was used for

detection of NDV. 20 μL of allantoic fluid was loaded in for samples. NDV-GFP was loaded as a control. MW used was the PageRuler™ Plus Prestained Protein Ladder (Thermo Scientific).

FIG. **11** shows graphs of results on protection from weight loss in NDV-COVID-19 vaccinated hamsters challenged with SARS-CoV-2. Groups of eight Syrian Golden hamsters were anaesthetized with inhalation isoflurane and administered 1E7 PFU/animal of recombinant NDV-GFP, NDV-FLS, or NDV-PFS via the intranasal (IN) route. For the prime/boost groups, 28 days following the initial vaccine administration, hamsters were administered a second dose of the homologous vaccine (1E7 PFU/animal by IN route). At 28 days post-prime or 28 days post-prime/boost, hamsters were moved into a CL-3 facility, anaesthetized with inhaled isoflurane and infected SARS-CoV-2. Challenge dose: Alpha variant @ 8.5E4 PFU/animal by IN, Ancestral (Wuhan) @ 1E5 PFU/animal by IN. After recovery from anesthetic hamsters were monitored daily throughout the course of infection. Body weights of hamsters were recorded daily. Error bars represent mean+/−SEM.

FIG. **12** shows graphs depicting SARS-CoV-2 viral RNA copies in the lung and nasal turbinates of vaccinated and challenged Syrian hamsters. At 5 days post challenge with Alpha variant @ 8.5E4 PFU/animal by IN or Ancestral (Wuhan) @ 1E5 PFU/animal by IN, vaccinated hamsters were euthanized and viral RNA copies in the lung and nasal turbinates quantified by qRT-PCR. A standard curve produced with synthesized target DNA was run with every plate and used for the interpolation of viral genome copy numbers. Viral RNA levels are reported as genome copy number. Error bars represent mean+/−SEM. Differences in the magnitude of virus copy number were assessed by Kruskall-Wallis test with Dunn's test for multiple comparisons.

FIG. **13** shows graphs depicting infectious SARS-CoV-2 in the lung and nasal turbinates of vaccinated and challenged Syrian hamsters. At 5 days post challenge with Alpha variant @ 8.5E4 PFU/animal by IN or Ancestral (Wuhan) @ 1E5 PFU/animal by IN, vaccinated hamsters were euthanized and infectious titers of SARS-CoV-2 in the lung and nasal turbinates determined. Homogenized tissue samples were serially diluted 10-fold in media and dilutions were then added to 96-well plates of 95% confluent Vero cells containing 504 of the media in replicates of three and incubated for five days at 37° C. with 5% CO_2. Plates were scored for the presence of cytopathic effect on day five after infection. Titers were calculated using the Reed-Muench method, converted to PFU after multiplying by 0.69 and reported as PFU/g of tissue.

DETAILED DESCRIPTION

Unless otherwise indicated, the definitions and embodiments described in this and other sections are intended to be applicable to all embodiments and aspects of the present disclosure herein described for which they are suitable as would be understood by a person skilled in the art.

In understanding the scope of the present disclosure, the term “comprising” and its derivatives, as used herein, are intended to be open ended terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but do not exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The foregoing also applies to words having similar meanings such as the terms, “including”, “having” and their derivatives. The term “consisting” and its derivatives, as used herein, are intended to be closed terms that specify the presence of the stated features, elements, components, groups, integers, and/or steps, but exclude the presence of other unstated features, elements, components, groups, integers and/or steps. The term “consisting essentially of”, as used herein, is intended to specify the presence of the stated features, elements, components, groups, integers, and/or steps as well as those that do not materially affect the basic and novel characteristic(s) of features, elements, components, groups, integers, and/or steps.

As used herein, the singular forms “a”, “an” and “the” include plural references unless the content clearly dictates otherwise.

Compositions

The term “Newcastle Disease Virus” (NDV), as used herein, includes without limitation, avian orthoavulavirus-1 (AOaV-1) and variants thereof. The genome of NDV is single-stranded, negative-sense, non-segmented RNA comprising six genes in the order 3′-NP-P-M-F-HN-L-S′ encoding six structural proteins: nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin-neuraminidase (HN), and a large polymerase protein (L). The NDV vector genome is packaged within an envelope (membrane), which is made of lipid bilayer, HN protein, and F protein. The M protein forms a grid-like array on the inner surface of the viral envelope. Inside the envelope the NP protein is tightly bound to the vector genome, forming a nucleocapsid complex. The L protein and P protein are loosely bound to nucleocapsid complex. NDV strains can be pathotypically categorized into three groups: velogenic (i.e. highly virulent), mesogenic (i.e. intermediate virulence), and lentogenic (i.e. non-virulent). Velogenic strains produce

severe nervous and respiratory signs, spread rapidly, and have high mortality rate in birds. Mesogenic strains cause coughing, affect egg quality and production, and have low mortality rate in birds. Lentogenic strains produce mild signs with negligible mortality in birds. Although NDV can infect humans, most cases are non-symptomatic, and only very rarely it causes a mild fever and/or conjunctivitis. A nucleic acid sequence that defines a strain as lentogenic is GGGAGACAGGGGCGCC (SEQ ID NO: 25), which is translated to GRQGRL (SEQ ID NO: 26) found in the F protein encoded by a nucleic acid sequence in Genbank accession number AF077761.1. A strain is mesogenic when there is a 3 amino acid change in the F gene, i.e. from GRQGRL to RRQRRF at amino acid positions 112, 115, and 117 in reference SEQ ID NO: 28. In some embodiments of this disclosure, the NDV vector is lentogenic. In some embodiments, the NDV vector comprises a nucleic acid comprising a nucleic acid sequence of SEQ ID NO: 25 or encodes the amino acid sequence of SEQ ID NO: 26. In some embodiments, the NDV vector is mesogenic. In some embodiments, the NDV vector comprises a nucleic acid comprising a nucleic acid sequence of SEQ ID NO: 23 or 27, or encodes the amino acid sequence RRQRRF (SEQ ID NO: 36).

As used herein, “transduction” of a cell by a viral vector means entry of the viral vector into the cell and transfer of genetic material into the cell by which nucleic acid incorporated in the viral vector is transferred into the cell.

The term “nucleic acid”, “nucleic acid molecule” or its derivatives, as used herein, is intended to include unmodified DNA or RNA or modified DNA or RNA. For example, the nucleic acid molecules of the disclosure can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically double-stranded or a mixture of single- and double-stranded regions. In addition, the nucleic acid molecules can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. The nucleic acid molecules of the disclosure may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. “Modified” bases include, for example, tritiated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus “nucleic acid molecule” embraces chemically, enzymatically, or metabolically modified forms. The term “polynucleotide” shall have a corresponding meaning.

As used herein, the term “polypeptide” encompasses both peptides and proteins, and fragments thereof of peptides and proteins, unless indicated otherwise. In one embodiment, the therapeutic agent is a polypeptide.

As used herein, the term “vector”, “viral vector”, “viral particle”, or “delivery vector”, and their derivatives, refer to a particle that functions as a nucleic acid delivery vehicle, and which comprises the viral nucleic acid (i.e., the viral vector genome) packaged within the particle. Viral vectors according to the present disclosure package a NDV vector genome. A “heterologous nucleic acid” or “heterologous nucleotide sequence” is a sequence that is not naturally occurring in the virus, i.e. a transgene. In general, the heterologous nucleic acid or nucleotide sequence comprises an open reading frame that encodes a polypeptide and/or a non-translated RNA.

The term “engineered Newcastle Disease Virus vector” or “engineered NDV vector” comprises an engineered (also interchangeably referred as “recombinant”) NDV vector genome packaged within an envelope, i.e. a DNA copy of the NDV antigenome comprised in an expression plasmid. The engineered NDV vector genome is capable of generating mRNA much like a native negative-sense NDV genome is capable of generating mRNA. The engineered NDV vector genome has a promoter, for example, an RNA promoter such as T7 immediately upstream of the 5′ end of the antigenome, or any suitable promoter known in the art, which drives expression of the virus RNA genome. The expression of a heterologous nucleic acid (transgene) such as one that encodes an immunogenic agent is driven by a typical NDV genome promoter. The T7 promoter, followed by 3 non-template guanines, is placed immediately upstream of the first nucleotide of the NDV vector genome. The engineered NDV vector genome described herein contains unique restriction sites for endonucleases such as XbaI and MluI for use in molecular biology techniques, for example, to facilitate efficient insertion of a heterologous nucleic acid. The skilled person would readily recognize endonuclease restriction sites such as XbaI and MluI. Engineered NDV vector genome can also contain an L289A mutation in the fusion (F) protein for enhanced fusion, a self-cleaving hepatitis delta virus (HDV) ribozyme sequence to ensure adherence to the “rule of six” by self-cleaving immediately at the end of the viral antigenomic transcript, and a T7 terminator sequence. An engineered NDV vector genome can also encode a F protein that has been mutated to contain a multi-basic cleavage site. The F protein and/or the HN protein of an engineered NDV vector genome can be substituted with the corresponding avian paramyxovirus (APMV) F protein and/or HN protein, or part thereof. Modification of F, HN or both, can be done using additional unique restriction endonuclease sites that flank these genes such as PacI, AgeI and AscI, which for example have been purposefully added in exemplified embodiments of this disclosure. When the substitution occurs in part, the resulting protein would be a chimeric protein, for example, a chimeric F protein and/or a chimeric HN protein containing sequence from NDV and APMV. The APMV can be APMV5.

The term “promoter,” as used herein, refers to a nucleotide sequence that directs the transcription of a gene or coding sequence to which it is operably linked.

The term “operably linked”, as used herein, refers to an arrangement of two or more components, wherein the components so described are in a relationship permitting them to function in a coordinated manner. For example, a transcriptional regulatory sequence or a promoter is operably linked to a coding sequence if the transcriptional regulatory sequence or promoter facilitates aspects of the transcription of the coding sequence. The skilled person can readily recognize aspects of the transcription process, which include, but not limited to, initiation, elongation, attenuation and termination. In general, an operably linked transcriptional regulatory sequence is joined in cis with the coding sequence, but it is not necessarily directly adjacent to it.

A “segment” of a nucleotide sequence is a sequence of contiguous nucleotides. A segment can be at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 85, 100, 110, 120, 130, 145, 150, 160, 175, 200, 250, 300, 350, 400, 450, 500 or more contiguous nucleotides.

A “fragment” of an amino acid sequence is a sequence of contiguous amino acids. A segment can be at least about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 75, 85, 100, 110, 120, 130, 145, 150, 160, 175, 200, 250, 300, 350, 400, 450, 500 or more contiguous amino acids.

The presence of the NDV vector genome can be tracked by a marker. In another embodiment, the NDV vector genome further comprises a nucleotide sequence encoding a marker. In another embodiment, the marker comprises GFP.

A “therapeutic agent” can be an agent that can alleviate or reduce symptoms that result from an absence or defect in a protein in a cell, tissue or subject. In addition, a “therapeutic agent” can be an agent that otherwise confers a benefit to a subject, e.g., anti-disease effects or improvement in survivability upon exposure to a causative agent of an infectious. A “therapeutic agent” can be a polypeptide, a therapeutic protein, an antigen, an antibody, or an antigen binding fragment. The antibody can be a monoclonal, polyclonal, chimeric, humanized antibody, or a fragment thereof, or a combination thereof. The antigen binding fragment is a Fab, Fab′, F(ab′)2, scFv, dsFv, ds-scFv, dimer, minibody, diabody, or multimer thereof or bispecific antibody fragment, or a combination thereof. A “therapeutic agent” can be an immunogenic agent.

The term “immunogenic agent” as used herein refers to a molecule that can elicit an immune response in a subject. The immunogenic agent can be an antigenic molecule such as a polypeptide that can induce, for example, humoral and/or cellular response, by

activating B cells for the production of antibodies, CD4+ T cells for helper cell functions, and CD8+ T cells for their cytotoxic functions. An immunogenic agent can be encoded by a heterologous nucleic acid comprised in the engineered NDV vector or vaccine of the present disclosure. An immunogenic agent can be a protein or fragment thereof from an infectious agent for a disease, for example, such as influenza, SARS, MERS, or COVID-19.

SARS-CoV-2 is the causative agent of COVID-19. An immunogenic agent can be, for example, the spike protein (also referred as "spike") or fragment thereof of SARS-CoV-2. SARS-CoV-2 includes Variants of Concern (VoC) such as the South African B.1.351 variant (Peiris, M. and G. M. Leung, 2020). Other variants include variant B.1.1.7 having spike protein mutations delta69-70, delta144Y, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H; variant B.1.351 having spike protein mutations L18F, D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, and A701V; and variant B.1.351 2P having spike protein mutation L18F, D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, A701V, and KV986-987PP. The spike protein can be modified to enhance its stabilization. For example, proline mutations, such as two of F817P, A892P, A899P, A942P, K986P, and V987P, and in particular K986P and V987P (Hsieh, C.-L., et al., Science 2020), can be introduced to create a pre-fusion stabilized spike protein immunogen, however, when there is only 2 proline mutations, it is relatively unstable and difficult to produce in mammalian cells. The present inventors found that when all six prolines are introduced (i.e. when the engineered NDV expresses HexaPro (6 prolines)), version of prefusion stabilized spike, that retains the prefusion conformation of the spike protein, is retained and it shows higher expression than only two prolines. The six proline spike protein can also withstand heating and freezing better than the two prolines spike protein. In addition, the furin-cleavage site (RRAR) in the spike protein can be mutated to GSAS to render it furin-cleavage deficient, thereby increases its half-life. The immunogenic agent can be for priming and/or boosting an immune response against an antigen. Engineered NDV vectors of the present disclosure that express the spike protein include the constructs having the sequence in SEQ ID NO: 2-4, 18 or 19, with those comprising the proline mutations and/or deficient furin-cleavage site shown in SEQ ID NO: 18 and 19. In an embodiment, the engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence any one of SEQ ID NO: 2-4, 18, or 19. In an embodiment, the engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 18 or 19. In an embodiment, the immunogenic agent is a SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the SARS-CoV-2 spike protein is encoded by the nucleic acid sequence having at least 75%, 80%, 85%,

90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the nucleic acid sequence of SEQ ID NO: 8 or 17. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to a sequence of GenBank reference QHD43416.1 or QIZ15537.1, or variant B1.1.7 having spike protein mutations delta69-70, delta144Y, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, and L18F; variant B.1.351 having spike protein mutations D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, and A701V; or variant B.1.351 2P having spike protein mutations L18F, D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, A701V, and KV986-987PP. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to a sequence of GenBank reference QHD43416.1. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to a sequence of GenBank reference QIZ15537.1. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising any two mutations selected from the group consisting of F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the mutations are K986P and V987P. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising mutations F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising mutations 682-RRAR-685 to 682-GSAS-685, and any two mutations selected from the group consisting of F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the mutations are K986P and V987P. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising mutations 682-RRAR-685 to 682-GSAS-685, F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. The term “pharmaceutically acceptable” in referring to diluent, buffer, carrier, or excipient, as used herein, includes any

and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, that are physiologically compatible. Pharmaceutically acceptable diluent, buffer, carrier, or excipient includes sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The skilled person can readily recognize the use of such media and agents for pharmaceutically active substances. In one embodiment, the engineered NDV vector is comprised in a pharmaceutical composition that includes a pharmaceutically acceptable diluent, buffer, carrier, or excipient.

The present inventors have provided an engineered Newcastle Disease Virus (NDV) vector comprising a nucleic acid comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. The present inventors have further provided a vaccine comprising an engineered NDV vector having a nucleic acid that comprises at least one heterologous nucleic acid segment encoding an immunogenic agent operably linked to a promoter capable of expressing the segment in a host cell, and methods of treating or preventing a disease, for example, an infectious disease, with said vaccine or engineered NDV vector.

Accordingly, herein provided is an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27.

Also provided is an isolated nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the isolated nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27.

In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell.

In another aspect, also provided is an engineered chimeric NDV vector comprising a nucleic acid having a nucleic acid sequence encoding a L protein having a stabilizing segment, a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus. In an embodiment, the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. The stabilizing segment in L protein provides stability to molecular clones in a host cell such as a bacterial cell. In an embodiment, the L protein comprises a stabilizing segment. In an embodiment, the L protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence as set forth in SEQ ID NO: 11. In an embodiment, the stabilizing segment in the L protein comprises the sequence 1287-VSPYIHISNDSQRLFTEEGVKEGNVVYQQI-1316 (SEQ ID NO: 20). In an embodiment, the host cell is a bacterial cell.

The chimeric F protein is a chimeric with N-terminus APMV5 F protein and C-terminus NDV F protein, for example, NDV F protein from amino acid positions 501 to 553 (SEQ ID NO: 28; encoded by SEQ ID NO: 32, i.e. F gene in accession AF077761.1), which once incorporated into the chimeric protein become amino acid positions 494 to 546 in the chimeric protein, such as shown in SEQ ID NO: 12. In an embodiment, the chimeric F protein comprises at the C-terminus 53 amino acids of NDV F protein from amino acid positions 501 to 553 of SEQ ID NO: 28. In an embodiment, the chimeric F protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 12. In an embodiment, the chimeric HN protein comprises at the N-terminus 53 amino acids of NDV HN protein from amino acid positions 1 to 53 of SEQ ID NO: 34. In an embodiment, the chimeric HN protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 13. In an embodiment, the nucleic acid further comprises at least one heterologous

nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell.

In an embodiment, the therapeutic agent comprises a SARS-CoV-2 spike protein or a fragment thereof. In an embodiment, the SARS-CoV-2 spike protein is encoded by the nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the nucleic acid sequence of SEQ ID NO: 8 or 17. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of GenBank reference QHD43416.1 or QIZ15537.1, or variant B1.1.7 having spike protein mutation of one or more of delta69-70, delta144Y, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, and L18F; variant B.1.351 having spike protein mutation of one or more of D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, and A701V; or variant B.1.351 2P having spike protein mutation of one or more of L18F, D80A, D215G, delta241-243, R246I, K417N, E484K, N501Y, D614G, A701V, and KV986-987PP. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having a sequence of GenBank reference QHD43416.1. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of GenBank reference QIZ15537.1. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising any two mutations selected from the group consisting of F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising mutations F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41, comprising mutations 682-RRAR-685 to 682-GSAS-685, and any two mutations selected from the group consisting of F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6. In an embodiment, the SARS-CoV-2 spike protein comprises an amino acid sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41,

comprising mutations 682-RRAR-685 to 682-GSAS-685, F817P, A892P, A899P, A942P, K986P, and V987P at the positions corresponding to positions of SEQ ID NO: 6.

The engineered NDV vector of the present disclosure can activate an immune response which is useful for its use as an immunogenic composition, an oncolytic agent, or a vaccine. Accordingly, also provided is an immunogenic composition, an oncolytic agent, or a vaccine, wherein the immunogenic composition, oncolytic agent, or vaccine comprises an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In some embodiments, the oncolytic agent comprises an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

Also provided in the present disclosure is a pharmaceutical composition comprising an engineered NDV vector having a nucleic acid comprising a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, and a pharmaceutically acceptable carrier.

The engineered NDV vector, vaccine, immunogenic composition, or pharmaceutical composition described herein can be lyophilized without significant negative effects. In some embodiments, the engineered NDV vector, vaccine, immunogenic composition, or pharmaceutical composition is lyophilized. In some embodiments, the lyophilized engineered NDV vector, vaccine, immunogenic composition, or pharmaceutical composition is comprised in a solution comprising 1) 5% sucrose, 2) 5% sucrose and 5%

lodixanol, 3) 2.5% sucrose, 5% lactose, 1 peptone, 5 mM Tris-HCl, pH 7.6, or 4) 2.5% sucrose, 2.5% lodixanol, 5% lactose, 1% peptone, 5 mM Tris-HCl, pH 7.6, prior to lyophilization.

Nucleic acid and amino acid sequences described herein are set out in Table 1.

TABLE 1 Sequences SEQ ID NO:
TAATACGACTCACTATAGGGACCAAACAGAGAATCCGTGAGTTACGATAAAAGGCGAAGG
1; nucleic
AGCAATTGAAGTCGCACGGGTAGAAGGTGTGAATCTCGAGTGCGAGCCCGAAGCACAAAC
sequence of
TCGAGAAAGCCTTCTGCCAACATGTCTTCCGTATTTGATGAGTACGAACAGCTCCTCGCG
NDV-GFP
GCTCAGACTCGCCCCAATGGAGCTCATGGAGGGGGAGAAAAAGGGAGTACCTTAAAAGTA
Molecular
GACGTCCCGGTATTCACTCTTAACAGTGATGACCCAGAAGATAGATGGAGCTTTGTGGTA
Clone
TTCTGCCTCCGGATTGCTGTTAGCGAAGATGCCAACAAACCACTCAGGCAAGGTGCTCTC
AF077761.1_
ATATCTCTTTTATGCTCCCACTCACAGGTAATGAGGAACCATGTTGCCATTGCAGGGAAA
LaSota_Kan
CAGAATGAAGCCACATTGGCCGTGCTTGAGATTGATGGCTTTGCCAACGGCACGCCCCAG
R (with
TTCAACAATAGGAGTGGAGTGTCTGAAGAGAGAGCACAGAGATTTGCGATGATAGCAGGA
stabilizing
TCTCTCCCTCGGGCATGCAGCAACGGAACCCCGTTCGTCACAGCCGGGGCAGAAGATGAT
sequence in
GCACCAGAAGACATCACCGATACCCTGGAGAGGATCCTCTCTATCCAGGCTCAAGTATGG
L)
GTCACAGTAGCAAAAGCCATGACTGCGTATGAGACTGCAGATGAGTCGGAAACAAGGCGA
ATCAATAAGTATATGCAGCAAGGCAGGGTCCAAAAGAAATACATCCTCTACCCCGTATGC
AGGAGCACAATCCAACTCACGATCAGACAGTCTCTTGCAGTCCGCATCTTTTTGGTTAGC
GAGCTCAAGAGAGGCCGCAACACGGCAGGTGGTACCTCTACTTATTATAACCTGGTAGGG
GACGTAGACTCATACATCAGGAATACCGGGCTTACTGCATTCTTCTTGACACTCAAGTAC
GGAATCAACACCAAGACATCAGCCCTTGCACTTAGTAGCCTCTCAGGCGACATCCAGAAG
ATGAAGCAGCTCATGCGTTTGTATCGGATGAAAGGAGATAATGCGCCGTACATGACATTA
CTTGGTGATAGTGACCAGATGAGCTTTGCGCCTGCCGAGTATGCACAACTTTACTCCTTT
GCCATGGGTATGGCATCAGTCCTAGATAAAGGTACTGGGAAATACCAATTTGCCAGGGAC

CATTCCGAGGGGACCGTCCCCTCGGTAATGGCGAATGGGACGTCGACTGCTAACAAAGCC
CGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGG
GCCTCTAAACGGGTCTTGAGGGGTTTTTTGCTGAAAGGAGGAACTATA

Inventors have also engineered and rescued a chimeric NDV virus that has the F protein and HN protein from avian paramyxovirus 5 (APMV5) (SEQ ID NO: 9). F protein and HN protein are constituents of the NDV envelope, embedded within the lipid bilayer membrane. The inventors designed and produced this chimeric virus because the APMV5 F gene has a multi-basic cleavage site, which, without wishing to be bound by theory, can be useful for fusion with cells. Since APMV-5 is not pathogenic in chickens, the swapping of portion of APMV5 F protein with NDV F protein would broaden the use of this virus as an oncolytic agent in jurisdictions where there are restrictions imposed on avian pathogens, for example in the US by the authority of USDA/CDC. Specifically, for the NDV-APMV5 F-HN chimeric molecular clone sequence, NDV-APMV5 F is composed mostly of APMV5 but the last 53 amino acids are from NDV. NDV-APMV5 HN is composed mostly of APMV5 but the first 53 amino acids are from NDV.

Accordingly, also provided is an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence encoding a L protein comprising a stabilizing segment, a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus. In some embodiments, the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In some embodiments, the chimeric F protein comprises at the C-terminus 53 amino acid of NDV F protein from amino acid positions 501 to 553 of SEQ ID NO: 28. In some embodiments, the chimeric HN protein comprises at the N-terminus 53 amino acids of NDV HN protein from amino acid positions 1 to 53 of SEQ ID NO: 34. In some embodiments, the stabilizing segment comprises an amino acid sequence as set forth in SEQ ID NO: 20. In some embodiments, the stabilizing segment is encoded by a nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO: 35. In some embodiments, the L protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence as set forth in SEQ ID NO: 11. In some embodiments, the chimeric F protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the chimeric

HN protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the nucleic acid further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In some embodiments, the therapeutic agent comprises a SARS-CoV-2 spike protein. In some embodiments, the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41.

Methods and Uses

The term “infectious disease”, “transmissible disease” or “communicable disease”, and their derivatives, as used herein, refer to or describe a disease or disorder resulted from an infection, for example, caused by infectious agents including viruses, viroids, prions, bacteria, nematodes such as parasitic roundworms and pinworms, arthropods such as ticks, mites, fleas, and lice, fungi such as ringworm, and other macroparasites such as tapeworms and other helminths. Examples of infectious diseases include viral diseases such as viral hemorrhagic fevers such as Ebola and Marburg virus disease, gastroenteritis, dengue fever, West Nile fever, yellow fever, influenza, respiratory syncytial virus disease, Lassa fever, rabies, smallpox, cowpox, horsepox, monkeypox, Hantavirus pulmonary syndrome, Hendra virus disease, human immunodeficiency virus infection and acquired immunodeficiency disease syndrome, Hepatitis, Zika fever, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), Coronavirus disease 2019 (COVID-19), infectious bronchitis, infectious laryngotracheitis, Rift Valley fever, porcine epidemic diarrhea, porcine transmissible gastroenteritis, swine acute diarrhea syndrome, feline infectious peritonitis, African swine fever, classical swine fever, and bacterial diseases including drug resistant bacterial diseases such as tuberculosis and methicillin-resistant *Staphylococcus aureus* infection, and drug resistant parasitic diseases such as malaria. In an embodiment of this disclosure, the infectious disease is a viral disease or a bacterial disease. In an embodiment, the viral disease is viral hemorrhagic fever, gastroenteritis, dengue fever, West Nile fever, yellow fever, influenza, respiratory syncytial virus disease, Lassa fever, rabies, smallpox, cowpox, horsepox, monkeypox, Hantavirus pulmonary syndrome, Hendra virus disease, human immunodeficiency virus infection and acquired immunodeficiency disease syndrome, Hepatitis, Zika fever, SARS, MERS, COVID-19, infectious bronchitis, infectious laryngotracheitis, Rift Valley fever, porcine epidemic diarrhea, porcine transmissible gastroenteritis, swine acute diarrhea syndrome, feline

infectious peritonitis, African swine fever, or classical swine fever. In an embodiment, the viral hemorrhagic fever is Ebola or Marburg virus disease. In an embodiment, the bacterial disease is a drug resistant bacterial disease. In an embodiment, the drug resistant bacterial disease is tuberculosis, methicillin-resistant *Staphylococcus aureus* infection, or a drug resistant parasitic disease. In an embodiment, the drug resistant parasitic disease is malaria. In an embodiment, the infectious disease is COVID-19.

The term “cancer” and its derivates, as used herein, refers to a group of diseases comprising cells having abnormal cell growth and metastasized or the potential to metastasize, i.e. invade or spread to other parts of the body. For example, cancer includes but not limited to pancreatic cancer, kidney cancer such as renal cell carcinoma, urogenital cancer such as urothelial carcinomas, melanoma, prostate carcinoma, lung carcinomas such as non-small cell carcinoma, small cell carcinoma, neuroendocrine carcinoma, or carcinoid tumor, breast carcinomas such as ductal carcinoma, lobular carcinoma, or mixed ductal and lobular carcinoma, thyroid carcinomas such as papillary thyroid carcinoma, follicular carcinoma, or medullary carcinoma, brain cancers such as meningioma, astrocytoma, glioblastoma, cerebellum tumors, or medulloblastoma, ovarian carcinomas such as serous, mucinous, or endometrioid types carcinomas, cervical cancers such as squamous cell carcinoma in situ, invasive squamous cell carcinoma, or endocervical adenocarcinoma, uterine endometrial carcinoma such as endometrioid or serous and mucinous types carcinomas, primary peritoneal carcinoma, mesothelioma such as pleura or peritoneum mesothelioma, eye cancer such as retinoblastoma, muscle cancer such as rhabdosarcoma or leiomyosarcoma, lymphomas, esophageal cancer such as adenocarcinoma or squamous cell carcinoma, gastric cancers such as gastric adenocarcinoma or gastrointestinal stroma tumour (GIST), liver cancers such as hepatocellular carcinoma or bile duct cancer, small intestinal tumors such as small intestinal stromal tumor or carcinoid tumor, colon cancer such as adenocarcinoma of the colon, colon high grade dysplasia, or colon carcinoid tumor, testicular cancer, skin cancers such as melanoma or squamous cell carcinoma, or adrenal carcinoma.

The term “treating” and its derivatives, as used herein, refers to improving the condition associated with a disease, such as reducing or alleviating symptoms associated with the condition or improving the prognosis or survival of the subject. The term “preventing” and its derivatives, as used herein, refer to averting or delaying the onset of the disease, such as inhibiting or avoiding the advent of the disease, or vaccinated against the disease, or the lessening of symptoms upon onset of the disease, in the subject. The term “prophylactic” shall have a corresponding meaning.

The term “subject” as used herein refers to any member of the animal kingdom, optionally a mammal, optionally a human. In an embodiment, the subject is a mammal. In an embodiment, the subject is a human, a non-human primate, a rodent, a feline, a canine, an ovine, a bovine, a porcine, a caprine, an equine, a lupine, a vulpine, or a mustelid. In an embodiment, the subject is human. In an embodiment, the *Mustela* is a weasel, a polecat, stoats, a ferret or a mink. In an embodiment, the subject is a mink.

Accordingly, the present disclosure provides a method of treating or preventing a disease in a subject, comprising administering an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, and wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment. In an embodiment, the host cell is selected from the group consisting of a human, primate, murine, feline, canine, ovine, bovine, porcine, caprine, equine, lupine, vulpine, and *Mustela* host cell. In a further embodiment, the promoter is capable of expressing the at least one heterologous nucleic acid segment encoding the therapeutic agent in muscle, airway, or lung cells. In an embodiment, the therapeutic agent is any therapeutic agent as described herein. In an embodiment, the disease is any disease described herein.

The engineered NDV vector of the present disclosure is also useful for eliciting an immune response. According, also provided is a method for eliciting an immune response in a subject comprising administering an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic

acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, 19, 23, 27, or 42. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Also provided is use of an engineered NDV vector for eliciting an immune response in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Further provided is use of an engineered NDV vector in the manufacture of a medicament for eliciting an immune response in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A

or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Even further provided is an engineered NDV vector for use in eliciting an immune response, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment. wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

The ability of the engineered NDV vector of the present disclosure to activate an immune response is useful for its use as a vaccine or an immunogenic composition. Accordingly, also provided is a method for vaccination, the method comprises administering a vaccine comprising an engineered NDV vector having a nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment. wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is

SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Also provided is use of a vaccine comprising an engineered NDV vector for vaccinating a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Further provided is use of a vaccine comprising an engineered NDV vector in the manufacture of a medicament for vaccinating a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an

immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Even further provided is a vaccine comprising an engineered NDV vector for use in vaccinating a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the at least one heterologous nucleic acid segment encodes a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, 19, 23, 27, or 42. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Also provided is a method for administering an immunogenic composition in a subject, the method comprises administering an immunogenic composition comprising an engineered NDV vector having a nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike

protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Also provided is use of an immunogenic composition comprising an engineered NDV vector for eliciting an immune response in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, 19, 23, 27, or 42. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Further provided is use of an immunogenic composition comprising an engineered NDV vector in the manufacture of a medicament for eliciting an immune response in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 2, 3, 4, 18, or 19. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

Even further provided is an immunogenic composition comprising an engineered NDV vector for use in eliciting an immune response in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the therapeutic agent is an immunogenic agent. In an embodiment, the immunogenic agent is SARS-CoV-2 spike protein or fragment thereof. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence any one of SEQ ID NO: 2, 3, 4, 18, 19, 23, 27, or 42. In an embodiment, the immunogenic agent activates B-cells, CD4+ T-cells and/or CD8+ T-cells.

The engineered NDV vector can function as a delivery vehicle that delivers heterologous nucleic acid segment (“payloads”) encoding a therapeutic agent for treating or preventing a disease such as an infectious. In one embodiment, the infectious disease is selected from the group consisting of viral diseases such as viral hemorrhagic fevers, Ebola, Marburg virus disease, gastroenteritis, dengue fever, West Nile fever, yellow fever, influenza, respiratory syncytial virus disease, Lassa fever, rabies, smallpox, cowpox, horsepox, monkeypox, Hantavirus pulmonary syndrome, Hendra virus disease, human immunodeficiency virus disease and acquired immunodeficiency disease syndrome, Hepatitis, Zika fever, optionally Ebola or Marburg virus disease, Severe Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), Coronavirus disease 2019 (COVID-19), and bacterial diseases including drug resistant bacterial diseases such as tuberculosis and methicillin-resistant *Staphylococcus aureus* infection, and drug resistant parasitic diseases such as malaria. In an embodiment, the infectious disease is COVID-19.

The immune response can be independent of expression of a therapeutic agent such as an immunogenic agent. For example, the engineered NDV vector disclosed herein can activate

NK cells in a subject bearing tumour. In some embodiments, the immune response comprises activation of NK cells. In some embodiments, the activation of NK cells comprises production of CD69, PD-L1, Granzyme B and/or IFNgamma. Such an immune response is useful for the treatment of, for example, cancer, such that the engineered NDV vector of the present disclosure is also useful as an anti-cancer agent. According, also provided is a method of treating cancer in a subject, comprising administering an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, or 10.

Also provided is use of an engineered NDV vector for treating cancer in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, or 10.

Further provided is use of an engineered NDV vector in the manufacture of a medicament for treating cancer in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, or 10.

Even further provided is an engineered NDV vector for use in treating cancer in a subject, wherein the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, or 10.

In some embodiments, the engineered NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% A or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27. In some embodiments, the cancer is pancreatic cancer, kidney cancer such as renal cell carcinoma, urogenital cancer such as urothelial carcinomas, melanoma, prostate carcinoma, lung carcinomas such as non-small cell carcinoma, small cell carcinoma, neuroendocrine carcinoma, or carcinoid tumor, breast carcinomas such as ductal carcinoma, lobular carcinoma, or mixed ductal and lobular carcinoma, thyroid carcinomas such as papillary thyroid carcinoma, follicular carcinoma, or medullary carcinoma, brain cancers such as meningioma, astrocytoma, glioblastoma, cerebellum tumors, or medulloblastoma, ovarian carcinomas such as serous, mucinous, or endometrioid types carcinomas, cervical cancers such as squamous cell carcinoma in situ, invasive squamous cell carcinoma, or endocervical adenocarcinoma, uterine endometrial carcinoma such as

endometrioid or serous and mucinous types carcinomas, primary peritoneal carcinoma, mesothelioma such as pleura or peritoneum mesothelioma, eye cancer such as retinoblastoma, muscle cancer such as rhabdosarcoma or leiomyosarcoma, lymphomas, esophageal cancer such as adenocarcinoma or squamous cell carcinoma, gastric cancers such as gastric adenocarcinoma or gastrointestinal stroma tumour (GIST), liver cancers such as hepatocellular carcinoma or bile duct cancer, small intestinal tumors such as small intestinal stromal tumor or carcinoid tumor, colon cancer such as adenocarcinoma of the colon, colon high grade dysplasia, or colon carcinoid tumor, testicular cancer, skin cancers such as melanoma or squamous cell carcinoma, or adrenal carcinoma. In an embodiment, the cancer is an ovarian cancer.

The use or administration of an engineered NDV vector to a subject comprises ingestion, instillation such as intranasally, inhalation such as via aerosol, or injection. The route of injection includes but is not limited to intradermal, subcutaneous, intramuscular, intravenous, intraosseous, intraperitoneal, intrathecal, epidural, intracardiac, intraarticular, intracavernous, intravitreal, intracerebral, intracerebroventricular, intratracheal or intraportal. In an embodiment, the engineered NDV vector is administered or used intravenously, intranasally, intratracheal, intramuscularly, or via aerosol. In an embodiment, the engineered NDV vector is administered or used intranasally. In an embodiment, the engineered NDV vector is administered or used intramuscularly. In an embodiment, the engineered NDV vector is delivered to muscle, airway, or lung cells or tissues.

The present disclosure further provides a method of producing a protein in vivo in a subject, comprising delivering or introducing into the subject an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a protein operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27.

In addition, the present disclosure provides a method of producing at least one protein in vitro in a host cell, comprising introducing into the host cell an engineered NDV vector comprising a nucleic acid having a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a protein operably linked to a promoter capable of expressing the segment in a host cell, and wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein. In an embodiment, the protein is any protein described herein. The skilled person can readily recognize the suitable production or manufacturing methods for producing proteins such as therapeutic agents using the engineered NDV vector as described herein. In an embodiment, the nucleic acid comprises a nucleic acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27.

Also provided is a method for selecting a stable engineered NDV vector genome. Inventors have developed a visual screening tool for selecting stable engineered clones based on their growth pattern on Luria-Bertani (LB) plates. When cloning transgenes (e.g. viral antigen for vaccine purposes) into the NDV genome and screening for colonies that contain the full-length NDV genome plasmid with the correct insert, the transformed bacteria often grow as both large and small colonies. The large colonies are visible after 16 hours whereas the smaller colonies need to grow for at least 24 hours before they are large enough to inoculate a liquid culture. The large colonies often contain mutated NDV genome plasm ids, whereas the small colonies invariably contain stable NDV clones and are thus selected for growth in liquid culture. Accordingly, also provided is a method for selecting an engineered NDV vector genome comprising a stabilizing segment in L gene, the method comprises:

- a) growing bacterial cells comprising an engineered NDV vector genome plasmid in growth medium broth;
- b) growing the bacterial cells on an agar-growth medium, wherein the agar-growth medium comprises a selection agent;
- c) identifying small bacterial cell colonies having about 0.5 mm to about 1 mm in diameter after at least 24 hours of growth;
- d) repeating step a) to step c) two to nine times to enrich for small bacterial cell colonies; and

- e) isolating the engineered NDV vector genome from the small bacterial cell colonies,
- wherein the small bacterial cells colonies comprise stable engineered NDV vector genome having the stabilizing segment in L gene.

In an embodiment, the growth medium broth is a Luria Bertani (LB) broth. In an embodiment, the agar-growth medium is agar-Luria Bertani (LB). In an embodiment, the selection agent is an antibiotic. In an embodiment, the antibiotic is kanamycin. In an embodiment, the stabilizing segment comprises an amino acid sequence as set forth in SEQ ID NO: 20. In an embodiment, the stabilizing segment is encoded by a nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO: 35. In an embodiment, the stable engineered NDV vector genome encodes a full-length L protein (SEQ ID NO: 11). In an embodiment, the bacterial cells are *E. coli*. In an embodiment, the *E. coli* is an *E. coli* strain Stellar, NEBStable, or GT116.

The following non-limiting Examples are illustrative of the present disclosure:

Example 1A. Development of NDV-FLS and NDV-A19S Immunogens Using Engineered Newcastle Disease Virus Vectors Expressing SARS-CoV-2 Spike Proteins

Materials and Methods

Engineered NDV Vector

The full-length cDNA genome of lentogenic NDV LaSota strain was synthetically designed based on accession AF077761.1 to contain a GFP reporter gene and essential NDV-specific RNA transcriptional signals, flanked by a 5′ XbaI site and a 3′ MluI site at position 3143 nucleotide between the P and M genes. Unique restriction sites between the P gene and the M gene were chosen because transgenes expressed between these sites are highly expressed and these restriction sites do not interfere with the stability of the recombinant virus. A leucine to alanine mutation at position 289 was also introduced into the Fusion gene. To construct recombinant NDV expressing SARS-CoV-2 Spike protein, forward 5′GCACCGAGTTCCCCCTCTAGATTAGAAAAAATACGG GTAGAACCGCCAC-3′ (SEQ ID NO: 21) and reverse 5′GTTGGACCTTGGGTAC GCGTTTATCAGGTGTAGTGCAGCTTCAC-3′ (SEQ ID NO: 22) primers were used to amplify human codon optimized SARS-CoV-2 full length spike protein. Additionally, a 19 amino acid truncated form of the Spike protein (SΔ19) was amplified using the above forward primer (SEQ ID NO: 21) and a reverse 5′G

TTGGACCTTGGGTACGCGTTTATCATCAGCAGCAAGAGCCGCAAGAACAAC-3′ (SEQ ID NO: 24). Infusion Cloning™ was used to insert transgenes into the NDV backbone according to the manufacturer's protocol (Takara Bio USA), with the 5′ end of the primer including 15 bp of homology with each end of the linearized vector including the XbaI or MluI sites. Viruses were rescued from cDNA, amplified and purified using methods described previously (Santry, L. A. et al., 2017) and confirmed by RT-PCR and sequencing.

DF-1 Infection Protocol

DF-1 cells (ATCC CRL-12203) were seeded into 6-well plates at 1.5×10^{6} cells/well in 1 mL of DMEM supplemented with 2% bovine calf serum (BCS) and 5% allantoic fluid. After adherence, the cells were infected with either NDV-FLS, -Δ19S or -GFP at MOI of 1 and 10 in replicate plates. The plates were incubated at 37° C. One day post infection, the replicate plates were observed under an inverted phase contrast microscope to examine and document cytopathic effect (CPE) with photographs. Subsequently, one set of replicate plates was collected for protein extraction and Western blot analysis, and the second set of replicate plates was used for immunofluorescence assay (IFA).

Immunofluorescence Assay

Approximately 1 day post infection, old media were removed and cells were rinsed twice with phosphate-buffered saline (PBS). Cells were then fixed in 4% paraformaldehyde (PFA) for 15 minutes at room temperature (RT). After fixation, cells were washed three times with PBS-T (PBS-1% tween) for 5 minutes each. The cells were then permeabilized in 0.1% NP-40 for 10 minutes at RT followed by three washes with PBS-T for 5 minutes each. Subsequently, cells were blocked in blocking buffer [5% (v/v) normal goat serum in PBS-T] either for one hour at RT or overnight at 4° C. After blocking, cells were incubated in primary mouse anti-NDV (NBP2-11633; Novus Biologicals) diluted 1:2000 in blocking buffer for one hour at RT (or overnight at 4° C.). Following the primary antibody incubation, cells were washed three times with PBS-T for 5 minutes each and then incubated with secondary goat-anti-mouse-488 (Invitrogen, ThermoFisher) diluted in 1:1000 in PBS-T for one hour at RT in the dark. Following secondary antibody incubation, cells were once more washed 3 times with PBS-T for 5 minutes each. After the final wash was removed, PBS-T was added to keep cells submerged under solution, and cells were imaged using an Axio observer inverted fluorescent microscope.

SDS-PAGE (Denaturing) and Western Blot Analysis

Infected DF-1 cells were washed with PBS and lysed in radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1× protease inhibitor cocktail) for 30 min on ice. Following lysis, cell lysates were centrifuged at 10,000×g for 15 min at 4° C. The supernatants were transferred to a new collection tube and debris was discarded. Protein amount in the supernatants were quantified using the Pierce BCA Protein Assay Kit (ThermoFisher) according to the manufacturer's instructions. For SDS-PAGE, cell lysates (mixed with 6× loading dye containing and 30% β-mercaptoethanol) were heated at 95° C. for 10 min to denature proteins, followed by cooling on ice. Protein, with amounts ranging from 5 µg to 70 µg depending on experiment, were loaded into wells of 4% stacking/12% resolving gels. The same protein amount of each sample was loaded within each experiment. Proteins were resolved at 120 V for 1.5 h in running buffer (0.025 mM Tris-base, 0.192 M glycine, 0.1% SDS), followed by semi-dry transfer to a 0.2 µm PVDF membrane for 30 min using the BioRad Trans-Blot Turbo Transfer System and BioRad proprietary buffer (BioRad Trans-Blot Turbo RTA Mini PVDF Transfer Kit). Following transfer, the rest of the protocol was performed as previously described (Pham P H et al., 2020). All wash steps were performed with PBS-T. The primary antibodies were either the mouse anti-NDV antibody (dilution: 1:5000; NBP2-11633; Novus Biologicals), rabbit anti-SARS spike protein antibody (dilution: 1:1000; NB100-56578; Novus Biologicals), or mouse anti-beta actin antibody (diluted 1:1000; MA5-15739; ThermoFisher). Primary antibodies were incubated overnight at 4° C. The secondary antibodies were either goat anti-rabbit or goat anti-mouse IgG conjugated to horseradish peroxidase (diluted 1:2000; ThermoFisher). Secondary antibodies were incubated for 1 to 3 h at RT. Protein was detected using the Pierce SuperSignal West Pico PLUS Chemiluminescent Substrate (ThermoFisher) and a BioRad ChemiDoc MP Imaging System (BioRad Image Lab 6.0.1. software).

Determination of Mean Death Time (MDT)

The MDT was determined for three viruses: NDV-FLS, -SΔ19, and -GFP. The virus stocks were equalized to the starting titre of 6.14×10^{6} FFU/mL. Each virus was diluted in a 10-fold 1 mL serial dilution series from 10^{-1} to 10^{-8} in PBS. To determine the MDT, virus dilutions from 10^{-4} to 10^{-8} were chosen to be inoculated into SPF eggs (Canadian Food Inspection Agency) at 9 to 11 days of embryonation. For each of the three viruses, a total of 50 eggs were used for two replicate MDT experiments (25 eggs per replicate), which were done in the same day but separated by 3 to 4 hours between replicates. Of the 25 eggs in each replicate MDT experiment, five replicate eggs received 100 µL of 10^{-4} diluted virus, five

received 100 µL of 10^{-5} diluted virus, five received 100 µL of 10^{-8} diluted virus, five received 100 µL of 10^{-7} diluted virus and five received 100 µL of 10^{-8} diluted virus. For the entire MDT experiment involving all three viruses, a total of 150 eggs were used. After virus inoculation, the eggs were incubated for up to 7 days and checked and scored twice daily for embryo mortality. Allantoic fluid was collected from dead embryos to check for presence of NDV by hemagglutination assay (HA). If no MDT was reached by the end of the experiment (7 days post inoculation), then HA was performed on allantoic fluid collected from eggs inoculated with the virus dilution containing the highest virus amount (10^{-4}) to confirm presence of NDV in eggs containing embryos that did not die (as defined by the AVIS Consortium, see http://www.fao.org/ag/againfo/programmes/en/empres/gemp/avis/A160-newcastle/mod0/0344-mdt-tests.html).

Hemagglutination Assay (HA)

For the HA, allantoic fluid (from eggs inoculated with NDV) was diluted in a 2-fold 100 µL serial dilution series from 2^{-1} (e.g. 50 µL of allantoic fluid and 50 µL of PBS) to 2^{-7} in PBS, in duplicate wells of a 96-well V-bottom plates. At the last dilution of 2^{-7}, after mixing, 50 µL of the mixture was discarded, leaving 50 µL remaining in these wells and the wells of the other dilutions. The above procedure was repeated for PBS alone and for allantoic fluid from uninfected control eggs; these served as negative controls for the HA. Once serial dilution was completed, 50 µL of 1% chicken red blood cells (diluted in PBS) was added to each well. The plates were incubated at RT for 45 min followed by scoring of the plates and documentation by photographs.

Rescue of SARS-CoV-2 Spike Protein Pseudotyped Lentiviral Particles

HEK 293T (human kidney cells, ATCC CRL-11268) cells grown in DMEM with 10% FBS and 1% penicillin/streptomycin were seeded in a 10 cm cell culture dish so that they would be 60-70% confluent the following day. 16-24 h post-seeding, cells were transfected using PolyJet™ Reagent (SignaGen Laboratories) in a 1:1 ratio of reagent-to-DNA with 6.7 µg of each of the following plasm ids: pSin-EF1α-luciferase, psPAX2 (Didier Trono; Addgene plasmid #12260; http://n2t.net/addgene:12260; RRID:Addgene_12260), and pCASI-SARS-CoV-2-Spike-Δ19. The following day the media was changed to fresh complete media. Starting at 48 hours post-media change, lentivirus was collected twice per day by changing media and replacing with complete media. Lentivirus was collected until 96 hours

post-media change for a total 5 collections. Lentivirus collections were pooled, filtered through a 0.45 μm PES filter and frozen as aliquots at −80° C.

Assessment of Luciferase Activity

1.25×10^4 HEK293T-hACE2 cells (Dr. Paul Spagnuolo, University of Guelph) were seeded per well in a 96-well plate and left to adhere overnight. The following day, media was removed and replaced with 40 μL of fresh complete media. Cells were then transduced with 60 μL of lentivirus, along with polybrene at a final concentration of 8 μg/mL. 60 hours post-transduction, luciferase activity was measured using the Pierce™ Firefly Luciferase Glow Assay Kit (Thermo Scientific) as per manufacturer's instructions. Luciferase readings were measured in white plates using an Enspire® Multimode Plate Reader (Perkin Elmer).

Statistical Analysis

All results were analyzed and plotted using GraphPad Prism 8 Software. Statistical significance was assessed using Mann-Whitney test, one-way analysis of variance (ANOVA), two-way ANOVA where appropriate.

Results

A fully synthetic molecular clone was engineered from lentogenic NDV (LaSota strain, Genbank accession AF077761.1) encoding a T7 promoter followed by three non-templated G's, unique XbaI and MluI restriction sites between the phosphoprotein (P) and the matrix (M) genes to facilitate transgene insertion, and a T7 terminator sequence. Also, an L289A mutation in the fusion (F) gene was also incorporated for enhanced fusion (Sergei, T. A et al 2000), and a self-cleaving hepatitis delta virus (HDV) ribozyme sequence was added to ensure adherence to the “rule of six” by self-cleaving immediately at the end of the viral antigenomic transcript (Kolakofsky, D., et al., 1998) (FIG. **1**A). Engineered NDV vectors expressing the full length human codon optimized SARS CoV-2 spike protein (NDV-FLS), spike protein with 19 amino acids deleted from its C-terminus (NDV-Δ19S), which has been shown to promote more efficient incorporation of spike protein into lentiviral (Johnson, M. C., et al 2020) and VSV (Fukushi, S., et al 2005) particles, and GFP (NDV-GFP), between the P and M genes (FIG. **1**A). Recombinant viruses were initially verified by immunofluorescence analysis of ribonucleoprotein (RNP) complex expression in NDV-FLS, NDV-119S and NDV-GFP infected DF-1 cells (FIG. **1**B) and by RT-PCR confirmation of spike gene insertion (FIG. **1**C). Western blot analysis of whole cell lysates from DF-1 cells infected with NDV-FLS or NDV-Δ19S showed robust expression of the full length spike

protein, and in the case of NDV-FLS infected cells, weak expression of the cleaved S1 receptor-binding subunit (FIG. **1**D). To investigate whether the spike protein expressed from NDV would be incorporated into the NDV virion, virus purified by gradient ultracentrifugation was subjected to Western blot analysis. As shown in FIG. **1**E, spike protein was incorporated into the virion of the NDV-FLS virus; however, spike protein lacking 19 amino acids from C-terminus was poorly incorporated into the NDV virion, and was only visible after over-exposure of the Western blot (FIG. **2**). Next, the spike protein was incorporated into the NDV virion to determine whether it would increase NDV infectivity in HEK 293T cells over-expressing human angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV-2. Using a 119S pseudotyped lentivirus neutralization assay, it was shown that neutralizing antibodies against SARS-CoV-2 spike do not affect NDV-FLS or NDV-Δ19S infection (FIG. **3**) indicating that incorporation of spike protein on the surface of the NDV virion does not alter infectivity or tropism of the vaccine.

To investigate whether expressing the SARS-CoV-2 spike protein, which retains its multi-basic cleavage site, would impact the fusogenic properties of NDV, DF-1 cells were infected with NDV-FLS, NDV-Δ19S or NDV-GFP and the number of multinucleated syncytia quantified. As shown in FIG. **1**F, all three viruses formed syncytia in the presence of trypsin. This shows that NDV expressing the spike protein is not more fusogenic than the parental NDV-GFP, suggesting that the spike protein, which has a multi-basic cleavage site, is not enhancing the fusogenicity of the NDV-FLS vaccine. However, NDV-expressing the FLS formed significantly smaller sized syncytia compared to either NDV-Δ19S or NDV-GFP (FIG. **1**G).

Finally, to confirm that engineering NDV to express FLS, Δ19S or GFP does not alter pathogenicity of NDV in its host species, mean death time (MDT) in embryonated chicken eggs was determined. All viruses had an MDT>110 hours and thus retained their lentogenic phenotype.

Taken together, these data demonstrate that NDV can be engineered to express the SARS-CoV-2 spike protein without altering the safety profile of this viral vector. Moreover, the full length spike protein is incorporated into the NDV virion more efficiently than the Δ19 truncated version. Inventors have herein provided engineered synthetic molecular clones that are advantageous over other molecular clones of NDV in that, for example, unique restriction sites introduced allow for efficient insertion of transgenes between the P and M genes in an orientation dependent manner as well as allow for the exchange of the F and HN genes, for example, with those from other paramyxoviruses.

Example 1B: Engineered Chimeric NDV Vector

Inventors have also engineered and rescued a chimeric NDV virus that has the F protein and HN protein from avian paramyxovirus 5 (APMV5) (SEQ ID NO. 4). F protein and HN protein are constituents of the NDV envelope, embedded within the lipid bilayer membrane. The inventors designed and produced this chimeric virus because the APMV5 F gene also has a multi-basic cleavage site, which, without wishing to be bound by theory, can be useful for fusion with cells. Since APMV-5 is not pathogenic in chickens the swapping of portion of NDV F protein with APMV5 F protein would broaden the use of this virus as an oncolytic agent in jurisdictions where there are restrictions imposed on avian pathogens, for example in the US by the authority of USDA/CDC. Specifically, for the NDV-APMV5 F-HN chimeric molecular clone sequence, NDV-APMV5 F is composed mostly of APMV5 but the last 53 amino acid are from NDV. NDV-APMV5 HN is composed mostly of APMV5 but the first 53 amino acids are from NDV.

Example 1C: Screening Tool and Method for Selecting Stable Engineered NDV Clones

Inventors have also developed a visual screening tool for selecting positive, stable engineered clones based on their growth pattern on Luria-Bertani (LB) plates. Normally, molecular clone of NDV is unstable in most strains of *E coli* (e.g. Stellar, DH5alpha, GT116) in so for a large portion of the polymerase gene (L) would be deleted resulting in the growth of large and small colonies. The large colonies invariably possessed deletions in the L gene. However, inventors showed that selection of small colonies (about 0.5 mm to about 1 mm in diameter after 24 h of growth) followed by multiple rounds of growth in LB broth followed by selection of small colonies on LB-Kanamycin plates resulted in selection of bacteria that formed small colonies and harbored stable molecular clones of NDV.

Example 2: Lyophilized NDV-FLS Retains its Infectivity

Materials and Methods

Triplicate samples of freshly harvested allantoic fluid containing NDV-FLS were aliquoted into 15 mL conical tubes in 1 mL volumes. Aliquots were either left untreated or adjusted to a final concentration of 5% sucrose, 5% sucrose/5% Iodixanol or mixed 1:1 with a solution containing 10% Lactose, 2% peptone, 10 mM Tris-HCl, pH 7.6. Using a LABCONCO Freeze Dry system Freezone®4.5, samples were immediately lyophilized at 44×10-3 MBAR and −52° C. for 16 hours. Lyophilized samples were stored at 4° C. for 48 hours before being

resuspended in 1 mL 5% sucrose/PBS and titered. Three 1 mL aliquots of allantoic fluid containing NDV-FLS were adjusted to 5% sucrose and frozen at −80° C. before titering. An additional three 1 mL aliquots were used to titer NDV-FLS in allantoic fluid immediately following harvest from eggs. All samples were titered by TCID50 on DF-1 cells as described above.

Results

Inventors demonstrated that NDV-FLS can be lyophilized to simplify storage and distribution requirements, without significant negative effects. Aliquots of NDV-FLS were brought to a final concentration of 5% sucrose, 5% sucrose/5% Iodixanol or mixed 1:1 with a solution containing 10% lactose, 2% peptone, 10 mM Tris-HCl, pH 7.6 and lyophilized for 16 h at −52° C. Two days later, samples were reconstituted and virus titer determined as shown in FIG. **4**. There was a ˜2-fold loss of infectivity when NDV-FLS is lyophilized in 10% lactose, 2% peptone, 10 mM Tris-HCl, pH 7.6 compared to virus frozen at −70° C.; however, given the convenience and greatly simplified storage and transportation requirements of a lyophilized vaccine, this reduction in infectivity is an acceptable tradeoff.

Example 3: Engineered NDV Vector as a Vaccine for COVID-19 in Mice

Methods and Materials

T Cell Responses

Male Balb/c mice were administered intranasally various doses of a vaccine comprising NDV that expresses the spike protein from SARS-CoV-2 (NDV-FLS). After 32 days, mice were boosted with the same dose of vaccine via the same route of administration. Five days after boost, the mice were euthanized and spike protein-specific CD8+ T cell and CD4+ T cell responses were quantified in the blood, spleen, bronchoalveolar fluid, and lung.

Intranasal Vs Intramuscular Administration

Male C57BL/6 or Balb/c mice were vaccinated either intranasally or intramuscularly with 5×10^{6} PFU NDV-FLS. At day 10 post-vaccine administration, a subset (n=4) of mice were terminally bled and the spike protein specific CD8+ and CD4+ T cell responses quantified. Mice were non-terminally bled prior to being boosted on day 28 with the same dose of

vaccine, and then bled again on days 5 and 10 post-boost, and spike protein specific CD8+ and CD4+ T cell responses quantified. In addition, at 10 days post-boost, bronchoalveolar lavage fluid was collected and measured for SARS-CoV-2 spike protein-specific IgA antibodies.

Results

Inventors show that administration of engineered NDV vector expressing SARS-CoV-2 spike protein to mice elicits humoral and cellular responses. SARS-CoV-2 spike protein-specific CD8+ T cell and CD4+ T cell responses were detected quantified and are shown in FIG. **5** and FIG. **6**, respectively. SARS-CoV-2 spike protein specific CD8+ and CD4+ T cell responses after intranasal or intramuscular administration were detected, quantified and compared, as shown in FIG. **7**. As well, robust anti-spike IgA antibodies were detected in the Balb/c strain of mice after intranasal delivery of the NDV-FLS spike using a primer (5×10^6 PFU) boost (5×10^6 PFU) regimen (see Table 2).

TABLE 2 Spike-specific IgA antibodies in bronchoalveolar lavage fluid IgA Treatment Dilution OD1 Dilution OD1 Dilution OD1 Dilution OD1 Dilution OD1 Dilution OD1 NDV-FLS I.N C57BL6 1:5 0.063 0 NDV-FLS I.N C57BL6 1:5 0.113 0 NDV-FLS I.N C57BL6 1:5 0.101 0 NDV-FLS I.N C57BL6 1:5 0.125 1:10 0.045 NDV-FLS I.N BalbC 1:5 0.124 1:10 0.074 1:20 0.041 NDV-FLS I.N BalbC 1:5 0.51 1:10 0.173 1:20 0.206 1:40 0.015 NDV-FLS I.N BalbC 1:5 0.236 1:10 0.142 1:20 0.09 1:40 0.075 1:80 0.083 1:160 0.036 NDV-FLS I.N BalbC 1:5 0.012 1:10 0 1:20 0.712 NDV-FLS I.M C57BL6 1:5 0.064 0 NDV-FLS I.M C57BL6 1:5 0.134 1:10 0.028 1:20 0.006 1:40 0.344 NDV-FLS I.M C57BL6 1:5 0 0 NDV-FLS I.M C57BL6 1:5 0.047 0 NDV-FLS I.M BalbC 1:5 0 0 NDV-FLS I.M BalbC 1:5 0 0 NDV-FLS I.M BalbC 1:5 0 0

Thus, inventors have demonstrated that the engineered NDV vector molecular clone designed to express the SARS-CoV-2 spike protein (NDV-FLS) leads to the production of spike protein-specific serum IgG and mucosal IgA antibodies as well as spike protein-specific T cells responses in mice administered with the NDV-FLS vaccine intranasally.

Example 4: Engineered NDV Vector Kills Tumor Cells In Vitro

The ability of engineered NDV vector of this disclosure in killing tumor cells was tested in vitro using cells from murine acute myeloid leukemia (AML) C1498 cell line. Cultured C1498 cells were treated with NDV-GFP-NY (Park M-S et al, PNAS 2006; Gao Q et al, J Virol 2008), mesogenic NDV-GFP-GM (which has a 3 amino acid change in the F gene that

makes it mesogenic (i.e. fusogenic), i.e. from GRQGRL to RRQRRF at amino acid positions 112, 115, and 117 in reference SEQ ID NO: 28, or lentogenic NDV-GFP-GL at varying MOI, and metabolic activity relative to untreated cells were measured by resazurin (cell proliferation) assay (FIG. **8**, left panel). The area under the curve in FIG. **8**, left panel was plotted in the graph on the right panel. These results show that mesogenic NDV-GFP-GM was significantly better than NDV-GFP-NY and lentogenic NDV-GFP-GL at killing C1498 cells in vitro.

Example 5: Engineered NDV Vector Stimulates NK Cells in Ovarian Tumor

Bearing Mice

The ability of engineered NDV vector to stimulate the immune system was tested in a model of ovarian tumor bearing mice (Russell et al., 2015). These tumor bearing mice were injected with phosphate-buffered saline mock control, adeno-associated virus (AAV) expressing thrombospondin-1 type I repeats (3TSR), AAV expressing Fc3TSR, or AAV expressing bevacizumab, in the absence or presence of engineered NDV-GFP-GM vector. The 3TSR is a glycoprotein with potent anti-angiogenic factor, which is used in cancer treatment; Fc3TSR is a stabilized form of this glycoprotein. Bevacizumab is a recombinant antibody targeting the vascular endothelial growth factor (VEGF), a pro-angiogenic protein. In this Example, 3TSR, Fc3TSR and bevazicumab were expressed by an adeno-associated virus, and used in combination with NDV-GFP delivered intravenously. Blood was obtained from the mice via retro-orbital bleeds 36 hours post NDV-GFP infection. Red blood cells were lysed, and remaining cells were stained via flow cytometry to analyze for markers indicative of immune stimulation. Over 90% NK cells were detected to express the early activation marker CD69 (FIG. **9**A) and over 20% NK cells were PD-L1+ in all groups injected with the engineered NDV-GFP vector, but there was negligible detection in its absence. Granzyme B+ and IFNy+NK cells were also detected in the engineered NDV-GFP vector group but not in its absence (FIG. **9**B). Together, these results demonstrated that NDV-GFP leads to the potent stimulation of NK cells in ovarian tumor bearing mice. NDV of the present disclosure is useful as an oncolytic agent.

Example 6: NDV-Prefusion Stabilized SARS-CoV-2 Spike (NDV-PFS) Protects Against SARS-CoV-2 in Hamsters

Prefusion Stabilized SARS-CoV-2 Spike (PFS) Expression

Expression of prefusion stabilized SARS-CoV-2 spike (PFS; SEQ ID NO: 41) in the allantoic fluid of embryonated eggs inoculated with NDV-PFS (SEQ ID NO: 4) was determined by Western immunoblotting. A 6% SDS-PAGE gel and rabbit anti-SARS-CoV-2 S1 (dilution: 1:1000; PA5-81795; ThermoFisher) was used for detection of SARS-CoV-2 spike (FIG. **10**; black arrow). A 10% SDS-PAGE gel and mouse anti-NDV ribonucleoprotein (dilution: 1:5000; NBP2-11633; Novus Biologicals) was used for detection of NDV. 20 μL of allantoic fluid was loaded in for samples. NDV-GFP was loaded as a control. MW used was the PageRuler™ Plus Prestained Protein Ladder (Thermo Scientific). These results showed robust expression of SARS-CoV-2 S1 from embryonated eggs inoculated with NDV-PFS, indicating the ability of this NDV platform for delivering a payload such as SARS-CoV-2 S1.

Protection from Weight Loss in NDV-COVID-19 Vaccinated Hamsters Challenged with SARS-CoV-2

The inventors next determined the effects of NDV-PFS vaccination on hamsters challenged with SARS-CoV-2. Groups of eight Syrian Golden hamsters (four male and four female, four to six weeks of age; Charles River) were anaesthetized with inhalation isoflurane and administered 1E7 PFU/animal of recombinant NDV-GFP, NDV-FLS, or NDV-PFS via the intranasal (IN) route. For IN vaccinations, anaesthetized hamsters were scruffed and vaccines were delivered in a 100 μL volume (q.s. with PBS) through the nares (50 μL per nare). Animals had their mouths held closed to ensure inhalation through the nose. For the prime/boost groups, 28 days following the initial vaccine administration, hamsters were administered a second dose of the homologous vaccine (1E7 PFU/animal by IN route). At 28 days post-prime or 28 days post-prime/boost, hamsters were moved into a CL-3 facility, anaesthetized with inhaled isoflurane and infected SARS-CoV-2 via the same IN method described above. Challenge dose: Alpha variant @ 8.5E4 PFU/animal by IN, Ancestral (Wuhan) @ 1E5 PFU/animal by IN. After recovery from anesthetic hamsters were monitored daily throughout the course of infection. FIG. **11** shows graphs of results of body weights of hamsters, which were recorded daily (error bars represent mean+/−SEM). These results showed that NDV-COVID-19 vaccination, in particular NDV-PFS vaccination, provided protection from weight loss in hamsters challenged with SARS-CoV-2, whether with the alpha variant or the ancestral strain.

Reduced SARS-CoV-2 Viral RNA Copies in the Lung and Nasal Turbinates of Vaccinated and Challenged Syrian Hamsters

The effects of NDV-COVID-19 vaccination on SARS-CoV-2 viral RNA copies in the lung and nasal turbinates in hamsters were determined. The hamsters were vaccinated and

challenged as above, and at 5 days post challenge with Alpha variant @ 8.5E4 PFU/animal by IN or Ancestral (Wuhan) @ 1E5 PFU/animal by IN, vaccinated hamsters were euthanized and viral RNA copies in the lung and nasal turbinates quantified by qRT-PCR. RNA was extracted with the QIAamp Viral RNA Mini kit (Qiagen) and reverse transcribed and amplified using the primers reported by the WHO and include E_Sarbeco_F1 (5′-ACAGGTACGTTAATAGTTAATAGCGT-3′; SEQ ID NO: 37) and E_Sarbeco_R2 (5′-ATATTGCAGCAGTA CGCACACA-3′; SEQ ID NO: 38) and probe E_Sarbeco_P1 (5′-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ-3′; SEQ ID NO: 39). A standard curve produced with synthesized target DNA was run with every plate and used for the interpolation of viral genome copy numbers. FIG. **12** shows graphs of viral RNA levels reported as genome copy number (error bars represent mean+/−SEM). Differences in the magnitude of virus copy number were assessed by Kruskall-Wallis test with Dunn's test for multiple comparisons. These results showed that NDV-COVID-19 vaccination, in particular NDV-PFS vaccination, reduced SARS-CoV-2 viral RNA copies in the lung and nasal turbinates of hamsters challenged with SARS-CoV-2, whether with the alpha variant or the ancestral strain.

Reduced Infectious SARS-CoV-2 in the Lung and Nasal Turbinates of Vaccinated and Challenged Syrian Hamsters

The effects of NDV-COVID-19 vaccination on infectious SARS-CoV-2 in the lung and nasal turbinates in hamsters were determined. The hamsters were vaccinated and challenged as above, and at 5 days post challenge with Alpha variant @ 8.5E4 PFU/animal by IN or Ancestral (Wuhan) @ 1E5 PFU/animal by IN, vaccinated hamsters were euthanized and infectious titers of SARS-CoV-2 in the lung and nasal turbinates determined. For infectious virus assays, thawed tissue samples were weighed and placed in 1 mL of minimum essential medium supplemented with 1% heat-inactivated fetal bovine serum (FBS) and 1×L-glutamine, then homogenized in a Bead Ruptor Elite Bead Mill Homogenizer (Omni International) at 4 m/s for 30 seconds then clarified by centrifugation at 1,500×g for 10 minutes. Samples were serially diluted 10-fold in media and dilutions were then added to 96-well plates of 95% confluent Vero cells containing 50 μL of the same medium in replicates of three and incubated for five days at 37° C. with 5% CO_2. FIG. **13** shows graphs of results from plates that were scored for the presence of cytopathic effect on day five after infection, and the titers were calculated using the Reed-Muench method, converted to PFU after multiplying by 0.69 and reported as PFU/g of tissue. These results showed that NDV-COVID-19 vaccination, in particular NDV-PFS vaccination, reduced infectious SARS-CoV-2 in the lung and nasal turbinates of hamsters challenged with SARS-CoV-2, whether with

the alpha variant or the ancestral strain. Together, these results showed that NDV-COVID-19 of the present disclosure, including NDV-PFS, is a useful platform for vaccine against COVID-19.

While the present disclosure has been described with reference to what are presently considered to be the preferred example, it is to be understood that the disclosure is not limited to the disclosed example. To the contrary, the disclosure is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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Claims

1. An engineered Newcastle Disease Virus (NDV) vector comprising a nucleic acid having a nucleic acid sequence that is at least 95% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1-5, 9, 10, 18, 19, 23, 27, or 42, wherein the nucleic acid comprises or further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell, wherein the nucleic acid comprises a nucleic acid sequence encoding an L protein having a stabilizing segment, and wherein the nucleic acid comprises XbaI and MluI restriction

endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

2. The engineered NDV vector claim 1, comprising a nucleic acid having a nucleic acid sequence that is at least 95% or 100% identical to the nucleic acid sequence of SEQ ID NO: 9, 10, 23, or 27.

3. The engineered NDV vector of claim 1, wherein the therapeutic agent comprises a SARS-CoV-2 spike protein.

4. The engineered NDV vector of claim 3, wherein the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41.

5. The engineered NDV vector of claim 1, comprising a nucleic acid having a nucleic acid sequence a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus.

6. An engineered Newcastle Disease Virus (NDV) vector comprising a nucleic acid having a nucleic acid sequence encoding an L protein having a stabilizing segment, a chimeric F protein, and a chimeric HN protein, wherein the chimeric F protein comprises avian paramyxovirus 5 (APMV5) F protein segment thereof at the N-terminus and an NDV F protein segment at the C-terminus, and wherein the chimeric HN protein comprises an NDV HN protein segment at the N-terminus and an AMPV5 HN protein segment at the C-terminus.

7. The engineered NDV vector of claim 6, wherein the nucleic acid comprises XbaI and MluI restriction endonuclease sites between nucleic acid sequence encoding phosphoprotein and matrix protein.

8. The engineered NDV vector of claim 6, wherein the stabilizing segment comprises an amino acid sequence as set forth in SEQ ID NO: 20, or comprises an amino acid sequence encoded by a nucleic acid comprising a nucleic acid sequence as set forth in SEQ ID NO: 35.

9. The engineered NDV vector of claim 6, wherein the chimeric F protein comprises at the C-terminus 53 amino acid of NDV F protein from amino acid positions 501 to 553 of SEQ

ID NO: 28, or the chimeric HN protein comprises at the N-terminus 53 amino acids of NDV HN protein from amino acid positions 1 to 53 of SEQ ID NO: 34.

10. The engineered NDV vector of claim 6, wherein the L protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence as set forth in SEQ ID NO: 11, the chimeric F protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 12, and/or the chimeric HN protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 13.

11. The engineered NDV vector of claim 6, wherein the NDV vector is lentogenic, and wherein the nucleic acid comprises a nucleic acid sequence of SEQ ID NO: 25.

12. The engineered NDV vector of claim 6, wherein the nucleic acid further comprises at least one heterologous nucleic acid segment encoding a therapeutic agent operably linked to a promoter capable of expressing the segment in a host cell.

13. The engineered NDV vector of claim 6, wherein the therapeutic agent comprises a SARS-CoV-2 spike protein.

14. The engineered NDV vector of claim 13, wherein the SARS-CoV-2 spike protein comprises an amino acid sequence having at least 95% identity to the amino acid sequence of SEQ ID NO: 6, 7, 29, 30, 31, or 41.

15. An immunogenic composition, oncolytic agent, or vaccine comprising the engineered NDV vector of claim 6.

16. A method for treating a disease, comprising administering to a subject the engineered NDV vector of claim 6.

17. A method of eliciting an immune response, comprising administering to a subject the engineered NDV vector of claim 6.

18. A method of treating cancer, comprising administering to a subject the engineered NDV vector of claim 1, wherein the NDV vector comprises a nucleic acid having a nucleic acid sequence that is at least 95% or 100% identical to the nucleic acid sequence of any one of SEQ ID NO: 1, 5, 9, 10, 23, or 27.

19. A method for selecting an engineered NDV vector genome comprising a stabilizing segment in L gene, the method comprises:

a) growing bacterial cells comprising an engineered NDV vector genome in a growth medium broth;

b) growing the bacterial cells on an agar-growth medium, wherein the agar-growth medium comprises a selection agent;

c) identifying small bacterial cell colonies having about 0.5 mm to about 1 mm in diameter after at least 24 hours of growth;

d) repeating step a) to step c) two to nine times to enrich for small bacterial cell colonies; and

e) isolating the engineered NDV vector genome from the small bacterial cells colonies,

wherein the small bacterial cells colonies comprise stable engineered NDV vector genome having the stabilizing segment in L gene.

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This is Exhibit AA referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Canadian Covid Care Alliance
Alliance canadienne pour la prévention et prise-en-charge de la covid

Concerns regarding the efficacy and safety for BNT162b2 mRNA coronavirus disease (COVID-19) vaccine through six months

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Summary of concerns

Efficacy

- Important limitations of the stated efficacy claims were not discussed
- Only the relative risk reductions were stated; absolute risk reduction metrics were not presented
- Integration of adult and adolescent cohorts with differing follow-up periods were presented without explanation
- Large number of discontinued or missing participants comparable to primary end-point event numbers
- Prior SARS-CoV-2 infections screened only in a subset of trial participants, and determined only by an antibody test with severe sensitivity limitations
- Cut-offs of the RT-PCR positivity tests were not reported; no confirmatory functional virology assays were performed
- Absence of systematic testing and unbiased testing framework for the detection of SARS-CoV-2-infected participants

Safety

- Trial participants were healthier than the average population
- Monitoring of adverse events were limited in time and scope
- Number of severe adverse events in the vaccine arm were much higher than the numerical reduction in severe COVID-19 cases between vaccine and placebo arms
- Superficial evaluation of the most clinically relevant end-point - survival; no independent assessment of the causes of death provided
- Cardiovascular adverse vaccine events are now widely recognized, yet no systematic monitoring of cardiovascular health was carried out
- Substantially higher number of solicited and unsolicited adverse events, most of which presented as COVID-19-like symptoms, in the vaccine arm yet study claims efficacy against symptomatic COVID-19
- Increase in cardiac-related deaths in the vaccine arm compared to placebo arm
- Inability to assess long term safety within the trial due to unblinding and participant crossover to the vaccine arm

Other concerns

- Multiple conflicts of interest of a large majority of study authors
- Multiple trial irregularities reported by Thacker *et al.* (1) published in the British Medical Journal

Article

We present several concerns regarding the recent article by Thomas *et al.* (2) on the efficacy and safety of the BNT162b2 mRNA coronavirus disease (COVID-19) vaccine, which was published in the New England Journal of Medicine (NEJM) on November 4, 2021. An abbreviated version of this letter was submitted to the NEJM on November 15, 2021 and declined for publication on November 29, 2021 due to limited space. The study assessed the BNT162b2 in individuals that were healthy or had stable chronic medical conditions and concluded that, "through 6 month follow up, despite a gradual decline in vaccine efficacy, BNT162b2 had a favorable safety profile and was highly efficacious at preventing COVID-19." We present numerous concerns regarding the reported safety and efficacy of this injection.

Efficacy

First, Thomas *et al.* (2) reported BNT162b2 efficacy as a relative risk reduction of contracting symptomatic reverse-transcriptase-polymerase chain reaction (PCR)-confirmed COVID-19 of 91.3% (77 vs 850 cases) and severe symptomatic PCR-confirmed COVID-19 of 96.7% (1 vs 30 severe cases). Thomas *et al.* (2) should have reported efficacy as an absolute risk reduction as per the communicating risks and benefits guidelines issued by the United States Food and Drug Administration (FDA) (3), which would have highlighted the modest absolute risk reductions provided by the vaccine in both symptomatic (3.7%) and severe symptomatic (0.7%) PCR-confirmed COVID-19.

Second, this analysis is the only published account of the BNT162b2 phase I – III trial efficacy outcomes among adults ≥16 years of age through six-month follow-up after immunization. In a trial amendment, a cohort of adolescents aged 12 to 15 years was added to the phase III study for which there was a shorter follow-up period. In this analysis, Thomas *et al*. (2) combined the two cohorts in providing efficacy outcomes after a six month follow up and departed from the initial analysis without providing a reasonable explanation for doing so. Given that vaccine efficacy wanes over time, by combining the older and younger cohorts, Thomas *et al.* (2) obfuscated the efficacy of the older group at six months. The authors should have provided efficacy outcomes for both groups and explicitly state the two reporting time periods in their conclusion.

Third, when discussing their findings, Thomas *et al.* (2) did not mention that a larger proportion of participants in the placebo group discontinued the trial compared to the vaccine group; 40% more after the first dose (271 vs 380 participants) and 63% more after the second dose (167 vs 273 participants). Discontinuations consisted mostly of "voluntary withdrawals", "no longer meeting the eligibility criteria" and "lost to follow-up." Additionally, there were a high number of participants missing from the CONSORT diagram between 2nd dose and the open-label period with more participants missing in the vaccine arm (1,258 vs 583 missing). These imbalances, which were in the order of the number of primary end-point events (77 and 850, for vaccine and placebo, respectively) call into question the reliability of these findings. Thomas *et al*. (2) should have disclosed the details related to the nature of these losses and discussed the impact they may have had on overall findings.

Fourth, Thomas *et al.* (2) used inappropriate tests when assessing current or prior infections due to severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). The authors screened 10,453 serum samples for COVID-19 infections up to 6 weeks prior to enrollment using the Roche Elecsys® Anti-SARS-CoV-2 antibody test, which tests for only the nucleocapsid protein of SARS-CoV-2 and has high sensitivity 14 days after infection when antibodies tend to peak (4). However, as antibody levels wane over time despite persisting immunity, it is unlikely that this test alone could identify prior immunity to SARS-CoV-2 or distinguish between prior immunity to other coronaviruses, which express similar proteins. Additionally, testing for the SARS-CoV-2 was done with the Cepheid Xpert Xpress SARS-CoV-2 RT-PCR rather than the gold-standard functional virology assay, looking for cytopathic effect in permissive cells. FDA specifications for PCR testing at that time the trial was conducted tended toward cycle thresholds beyond 20-30 cycles (5), which are now widely recognized as being unreliable in detecting an active COVID-19 infection (6-8). Given these limitations, Thomas *et al.* (2) should have used better screening for natural immunity, used a functional virology assay, and discussed the implications of these testing limitations in their findings.

Fifth, we noted an absence of systematic testing and an objective testing framework for the detection of SARS-CoV-2-infected participants. In this study, it was left to the discretion of the investigator to send a patient presenting with COVID-19-like symptoms for laboratory confirmation of SARS-CoV-2 infection, a task which would be particularly difficult given that reactogenicity events consisted principally of COVID-19-like symptoms (Thomas *et al.* (2), Figure S1). This lack of systematic testing introduced a concerning level of variability and subjectivity associated with the identification of both symptomatic cases and disease severity (9,10). Thomas *et al*. (2) should have discussed the implications of this lack of objective and systematic virological assessment on their study findings as well as presented data related to asymptomatic testing that was conducted at "selected sites." Overall, the emphasis on relative risk reductions, the combining efficacy outcomes from the adult and adolescent cohorts, the large number of people who were excluded from the analysis, and the use of inappropriate tests and lack of objective testing framework call into question the authors' conclusions regarding vaccine efficacy.

Safety

First, Thomas *et al.* (2) concluded their article by stating that BNT162b2 showed a "favorable safety profile," and in their abstract stated that "BNT162b2 continued to be safe and have an acceptable adverse-event profile." However, Thomas *et al.* (2) Figure S1 summarized solicited adverse events reported within 7 days of the first dose in the reactogenicity subset, which represented a mere 22% of the randomized population. A considerably higher rate of local and systemic adverse events was reported among vaccine recipients with a marked increase in adverse events with the second dose. The preponderance of systemic effects in both arms were COVID-19-like symptoms and occurred at higher rates than in the vaccine compared to the placebo group, despite the vaccine group having a higher number of identified symptomatic COVID-19 cases (77 vs 850, vaccine vs placebo, respectively). The very need for this trial is predicated on the importance and clinical relevance of eradicating COVID-19 symptoms. How is it then that such consistent increases in COVID-19-like symptoms among vaccine recipients are described as "favorable"?

Second, Thomas *et al*. (2) provided a descriptive analysis of vaccine safety. To better compare the benefits and the risks of this vaccine, we calculated absolute and relative risk reductions/increases (ARR/ARI and RRR/RRI, respectively) associated with the vaccine for efficacy events seven days after the second dose (i.e., corresponding to full vaccination for those in the vaccine group) and for safety events during the respective data collection period (starting with the first-dose). These calculations were based on the eligible population for each relevant safety and efficacy events without adjusting for surveillance time as that data was not published for safety events. A simple chi-square calculator was used to assess the significance of the difference in event numbers between groups (Table 1) (11).

Table 1. Differences in the number of efficacy and safety events in eligible populations[¥] reported in the 6-month update of the BNT162b2 mRNA Covid-19 vaccine

Event	BNT162b2 (n)	Placebo (n)	Absolute Difference (p-value)[?]	Absolute Risk Change* (%)	Relative Risk Change* (%)
Cases Adults and Adolescents 7 days after 2nd dose[$]	77	850	-773 (p<0.00001)	-3.7	-90.9
Any Unsolicited Treatment-Related Adverse Event Adults[#]	5,241	1,311	+3,930 (p<0.00001)	+17.9	+299.7
Any Severe Event Adults[/]	390	289	+101 (p=0.0001)	+0.5	+34.9
Severe Cases in Adults 7 days after 2nd dose[&]	1	23	-22 (p<0.00001)	-0.1	-95.7
Unsolicited Severe Adverse Events~ Adults Prevents daily routine activity or requires intervention or worse	262	150	+112 (p<0.00001)	+0.5	+74.6
Serious Adverse Event Adults[§] Requires hospitalization or results in permanent injury or death	127	116	+11 (p=0.5)	+0.05	+9.5
Deaths during placebo-controlled period [additional deaths during open-label period in vaccine recipients or placebo-only][%]	15 [+5]	14 [NR]	+1 [+5] (p=0.9)	+0.005	+7.1
Deaths due to cardiovascular events^	9	5	+4		

[¥] For the purpose of this table and in accordance with the terminology used in the study report, adult and adolescent populations are defined as ≥16 years old and 12-15 years old, respectively.
[?] Significance figures (p-values) estimated using chi-square calculator available at https://www.socscistatistics.com/tests/chisquare. P-values are without the Yates correction. This procedure was applied following the framework used by Classen (11) in his analysis of "All Cause Severe Morbidity" based on data from the initial reports of the vaccine Phase III trials
** Authors estimated vaccine efficacy using total surveillance time as denominator, however, as this value was unavailable for all the events analyzed, our calculations used the common statistical definition, i.e., number of events relative to total number of eligible patients for each event analysis reported[29] similar to previous analyses of this nature (11-30);*
[$] ≥7 Days after dose 2 among participants without evidence of previous infection
[#] Adverse events reported outside of the reactogenicity subgroup and assessed by the investigator as related to investigational product
[/] In calculations combining efficacy and safety events, the number of patients randomized that received any dose of vaccine or placebo was used as the study population in the statistical calculations, following the framework used by Classen (11) in his analysis of "All Cause Severe Morbidity". Differences in the total (event-incident) population (randomized vs efficacy vs safety) used as denominator are relatively small and are expected to have minimal impact on the relative differences between groups. Without access to individual patient data, these calculations were performed under the assumption that efficacy and safety events were non-overlapping
[&] ≥7 Days after dose 2; confirmed severe COVID-19 defined as PCR-positivity and "presence of at least one of the following: • Clinical signs at rest indicative of severe systemic illness (RR ≥30 breaths per minute, HR ≥125 beats per minute, SpO2 ≤93% on room air at sea level, or PaO2/FiO2 <300 mm Hg); • Respiratory failure (defined as needing high-flow oxygen, noninvasive ventilation, mechanical ventilation, or ECMO); • Evidence of shock (SBP <90 mm Hg, DBP <60 mm Hg, or requiring vasopressors); • Significant acute renal, hepatic, or neurologic dysfunction;• Admission to an ICU; • Death"
~ Severe (grade ≥3) adverse events were generally defined as those that interfere significantly with participant's usual function, those that affect daily living or require medical care; grade 4 events were generally defined as those that required emergency room visit or hospitalization
[§] Serious adverse events were defined as any untoward medical occurrence that, at any dose: a. Results in death; b. Is life-threatening; c.

Requires inpatient hospitalization or prolongation of existing hospitalization; d. Results in persistent disability/incapacity.
% Deaths during the open-label period were reported only in vaccine recipients, 3 participants in the BNT162b2 group and 2 in the original placebo group who received BNT162b2 after unblinding
^Those with reported cause of death due to: aortic rupture, arteriosclerosis, cardiac arrest, cardiac failure congestive, cardiorespiratory arrest, hypertensive heart disease, or myocardial infarction

Our findings showed that the increase in unsolicited adverse events in vaccine recipients, which included at least one adverse event up to 1 month post the second dose, was greater (RRI of 299.7% and ARI of 17.9%; p<0.00001) than the reduction in identified symptomatic COVID-19 cases observed in fully-vaccinated individuals for the duration of the trial (RRR of 90.9% and ARR of 3.7%; p<0.00001).

A similar pattern was observed for severe and serious adverse events. The study concluded that "vaccine efficacy against severe disease was 96.7%." However, our analysis showed that the vaccine was associated with a significant increase in severe adverse events defined as an adverse event that interferes significantly with daily activity or requires medical care (RRI of 74.6% and ARI of 0.5%; p<0.00001) and a numerical increase in serious adverse events, defined as any untoward medical occurrence that was life-threatening, required hospitalization or resulted in persistent disability up to 6 months (RRI of 9.5% and ARI of 0.05%; p=0.5) compared to placebo. These increases were greater than the reduction in severe COVID-19 cases observed in fully-vaccinated individuals for the duration of the trial (RRR of 95.7% and ARR of 0.1%; p=0.00002). When severe COVID-19 events were pooled with severe or serious adverse events to determine the likelihood of experiencing any severe event (11), there was an overall increase in severe events among vaccine recipients compared with placebo (RRI of 34.9% and ARI of 0.5%, p=0.0001). Given these findings, Thomas *et al.* (2) should have revised their conclusion to state, "the vaccine was associated with a concerning and clinically meaningful increase in severe events relative to placebo."

Third, Thomas *et al.* (2) conducted minimal monitoring of adverse events (12). Firstly, the solicited reactogenicity data was collected for only a small portion of trial participants (9,839/44,047 or 22.3%), for a limited 7 days after each dose, and for only a short pre-specified list of systemic and injection site reactions with no monitoring of sub-clinical effects. Secondly, unsolicited adverse events were collected for a mere 1 month and serious adverse events for only 6 months following the second dose. This means that severe vaccine related cardiac, neurological or immunological injuries that took more than a month to diagnose and were not considered serious, would not be reflected in the findings. Thirdly, unblinding and subsequent crossover of those on the placebo arm to the vaccine arm, will certainly attenuate any safety signals coming from this trial as well as preclude insights into long-term safety which were to be monitored for 2 years. Thomas *et al*. (2) should have commented on the implications their abbreviated monitoring schedule may have on safety underreporting as well as the implications of unblinding on short- and long-term safety outcomes. Given the increase in severe events (RRI of 34.9% and ARI of 0.5%) and cardiovascular deaths associated with the vaccine (n= 9 vs 5, vaccine vs placebo, respectively), The authors should have more closely monitored safety and provided a detailed discussion of the severe and serious adverse events along with a discussion of their potential long-term implications.

Fourth, given the inclusion of adolescents and "healthy participants who had stable chronic medical conditions" in the study population, we noted very little discussion of death, the most clinically

relevant end-point of this trial. Thomas *et al.* (2) Table S3 showed a slightly higher number of deaths in the vaccine group (n=15 vs n=14 in the placebo group during the blinded period). However, the manuscript text (Thomas *et al*. (2), page 7) stated that five additional deaths occurred in vaccine recipients after unblinding (two of which were initially allocated to the placebo group) for a total of 20 deaths in vaccine recipients. Thomas *et al.* (2) Table S4 also showed that although only 3 study deaths were attributed to COVID-19 or COVID-19 pneumonia (n=1 vs n=2, vaccine vs placebo, respectively) a total of 14 deaths were cardiovascular in nature (aortic rupture, arteriosclerosis, cardiac arrest, cardiac failure congestive, cardiorespiratory arrest, hypertensive heart disease) with the almost twice as many occurring in the vaccine arm (n=9 vs n=5, vaccine vs placebo, respectively). There is currently an abundance of real-world evidence to support an association between cardiovascular adverse events and the vaccines (13-17). Thomas *et al.* (2) reported that "none of these deaths were considered to be related to BNT162b2 by the investigators" without describing the objective framework of testing that allowed them to arrive at that conclusion or whether their findings were independently evaluated. Given the seriousness of these adverse events in an otherwise healthy population, Thomas *et al.* (2) should have provided a detailed description of how they arrived at their conclusion, these evaluations should have undergone independent assessment, and all ongoing study protocols investigating BNT162b2 should be immediately amended to include systematic short- and long-term clinical and sub-clinical monitoring of cardiovascular health. Overall, the increased rates of COVID-like symptoms, unsolicited adverse events as well as severe and serious adverse events in the vaccine compared to the placebo arm, as well as the net increase in deaths in vaccine recipients compared with those who were unvaccinated present serious concerns regarding the safety of these biological agents.

Conflicts of Interest

The disconnect between author conclusions, our analysis of the data, and the NEJM rejection of our letter to the editor led us to examine author disclosures for potential conflicts of interest (COI) (Table 2). Our analysis revealed multiple direct conflicts of interest. The article was supported by BioNTech and Pfizer, the corresponding author, Judith Absolon, and the senior author, Kathrin Jansen were employees of Pfizer and owned company stock, and the first author Stephen Thomas was a consultant to Pfizer. Of the 32 authors, 21 (66%) were employees of Pfizer or BioNtech and 26 (81%) had Pfizer/BioNtech-related conflict of interests. We also noted that one of NEJM's senior editors is also a co-principal investigator of the related Moderna-Vaccine COVE-trial (18,19).

Table 2. Conflicts of interest related to Pfizer/BioNTech

Title	Author
Corresponding author	Judith Absalon: Pfizer employment and stock holder
First author	Stephen Thomas: Pfizer consultancy
Last author	Kathrin Jansen: Pfizer employment and stock holder
Other 29 authors (66% employees, 81% had some COI)	Pfizer/ BioNTech employment and stockholder, n=15; Pfizer/ BioNTech employment (without stock) n=4; Pfizer grant/contract n=3; Pfizer clinical trial n=1; Other company consultancy n=1; No COI n=5

Conclusion

Our critique of the Thomas *et al*. (2) publication revealed multiple concerns regarding author claims of BNT162b2 safety and efficacy as well as a high number of direct conflicts of interest in the publication authors. These, coupled with multiple reports indicating that vaccine efficacy wanes within months of administration (20-23), reduced effectiveness of BNT162b2 with respect to emerging variants (24-26), record rates of serious adverse events (122,833) and deaths (17,128) reported in the US passive Vaccine Adverse Event Reporting System, VAERS by October 16, 2021, and problems with data integrity in the conduct of this trial reported recently by Thacker (1) in the British Medical Journal, raise further concerns regarding both the efficacy and safety of this agent. We did not find sufficient evidence to support use of these agents in the healthy adults studied or in specific unstudied demographics that are being mandated to comply with vaccination including the naturally immune, the frail elderly, those with multiple co-morbidities, the immunocompromised, and pregnant women. It also calls into question use in adolescents and children given that companion trials conducted in those populations suffered from similar design flaws, including underpowered in participant numbers and that recommendations for use were based on minimal safety follow up (27,28).

Conflicts of Interest

Byram W. Bridle received funding from the Ontario Government (COVID-19 Rapid Research Fund, Ministry of Colleges and Universities) and Government of Canada (Pandemic Response Challenge Program, National Research Council of Canada) to conduct pre-clinical research with COVID-19 vaccines
Ilidio Martins, none to disclose
Claudia Chaufan, none to disclose
Julian Northey, none to disclose
Niel A. Karrow, none to disclose
Steven Pelech is the majority shareholder and president and Chief Scientific Officer of Kinexus Bioinformatics Corporation, which has been developing serological tests for detection of antibodies against SARS-CoV-2 proteins and testing of drugs to inhibit SARS-CoV-2 replication
Bonnie Mallard, none to disclose
Christopher A. Shaw has been an expert witness in Vaccine Court twice
David Speicher, none to disclose
Ondrej Halgas, none to disclose
Deanna McLeod, none to disclose

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This is Exhibit BB referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

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Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Forcing People Into COVID Vaccines Ignores Important Scientific Data

thefederalist.com/2021/12/14/forcing-people-into-covid-vaccines-ignores-important-scientific-information/

December 14, 2021

Image CreditLisa Ferdinando / U.S. Army

The attacks on free speech and science are unrelenting. Academic publisher Elsevier's suppression of an article documenting the myocarditis risk of the COVID-19 vaccines, with no excuse or pretext offered, is incredible enough. Viewed alongside Twitter's censorship of the American Heart Association, YouTube's suppression of a panel discussion of vaccine mandates on Capitol Hill, and the Orwellian call by National Institutes of Health Director Francis Collins for critics of the government's COVID-19 policies to be "brought to justice," the trend is positively chilling.

Now more than ever, we need substantive debate about decisions that affect the health of hundreds of millions of people, including views counter to official positions. Instead, we have National Institute of Allergy and Infectious Diseases Director Anthony Fauci's absurd claim "I represent science" as proof of how one-dimensional our COVID-19 policymaking has become.

These are just a few examples of the wave of censorship that has accompanied COVID-19, uniting government bureaucracies with obedient news media, academia, scientific publishing, and powerful Big Tech companies. Above all, this concerted campaign suppresses all disagreement about topics including potential early treatments, the natural immunity of recovered individuals, and the safety and efficacy of COVID-19 vaccines. Differing viewpoints on these topics are swiftly labeled “disinformation,” but in fact represent principled dissent based on a large and growing body of scientific evidence.

Universal Vaccination Based on False Premise

In the case of COVID-19 vaccines, the censorship aims to stamp out any questions about a universal vaccination program that, it is now clear, was based on the false premise that low-risk individuals must get vaccinated to halt the spread of COVID-19 and end the pandemic. Almost a year into the global vaccination campaign – and starting long before omicron arrived – all the data stand in stark opposition to this belief.

Rapidly waning vaccine efficacy and COVID-19 surges in countries and regions with high vaccination rates – including Israel, the United Kingdom, Singapore, and now Europe, as well as high-vaccination U.S. states like Vermont – are evidence that vaccinated individuals can spread COVID-19 at rates comparable to the unvaccinated. Multiple studies have shown that viral load in vaccinated individuals with COVID-19 is the same as in the unvaccinated.

Most damning, reports regularly published by the British government show that for every age group from 30 years and up, vaccinated individuals are now actually more likely to test positive for COVID-19. In the case of the 40-59-year-old age group, in the latest report the rate is twice as high among the vaccinated.

Whether this is due to the physiological effects of the vaccines or to social factors – for example freer socializing by the vaccinated – the United Kingdom’s record-breaking surge across a mostly vaccinated population makes one thing clear: mass vaccination will not stop the pandemic. Similar surges fueled by breakthrough cases around the world tell the same story.

This is not disinformation but simply data, which everyone should be free to consider and discuss – even more so as it bears critically on the cost-benefit analysis individuals must make as they decide whether to receive the COVID-19 vaccine and subsequent boosters.

That’s because, whatever vaccine makers and government agencies may say, it is also clear that the COVID-19 vaccines are not without risks, which for some individuals extend to permanent life-altering injuries and even death. For individuals at high risk of severe

COVID-19 disease, the risks posed by vaccines may make sense, but for low-risk individuals, such as the vast majority of children, adolescents, and young adults of child-bearing age, the calculation is very different.

Discussing Risks Is not Disinformation

Any discussion of vaccine-related injuries and mortality is immediately labeled disinformation because it necessarily relies on the Vaccine Adverse Event Reporting System (VAERS), an imperfect legacy institution that allows anyone to file a report, conveniently enabling skeptics to dismiss the entire issue of vaccine risks as unfounded anecdote and fabrication. However, any responsible public health program should not take as its starting (and ending) point the assumption that the reports are all false, but instead consider the opposite: what if the numbers on VAERS are real – or even worse, represent substantial underreporting?

The numbers that we have are not reassuring. Since the COVID-19 vaccination program began last December, VAERS has recorded a total of more than 946,000 post-vaccination adverse events and almost 20,000 post-vaccination deaths. The largest daily death counts occurred within two days of vaccination, gradually subsiding with the length of time since the shot – a very strong temporal signal that there is a causal connection, not mere coincidence, behind these events.

The trend is corroborated by data from abroad: over the same period, the United Kingdom's Yellow Card system, equivalent to VAERS, has recorded 400,000 individual reports of adverse events following COVID vaccination, including more than 1,800 deaths.

Possible Underreporting of Adverse Events and Deaths

Moreover, counter to those who dismiss VAERS data as inflated, historical data suggests that vaccine-related adverse events and deaths are in fact underreported by a large margin. The Lazarus Report, funded by the U.S. Department of Health and Human Services, found that "fewer than 1% of vaccine adverse events are reported," and a 2015 study published in the scientific journal Vaccine acknowledged "known underreporting of adverse events to VAERS."

A Centers for Disease Control study analyzing VAERS reports from 1991 to 2001 warned that adverse events may be underreported. The U.S. Food and Drug Administration has admitted that it was incapable of tracking adverse events regarding the COVID-19 vaccines. In a letter to Pfizer dated August 23, 2021, the FDA stated, "the pharmacovigilance system that FDA is required to maintain … is not sufficient to assess these serious risks."

Why would doctors fail to report adverse events, including deaths, among recently vaccinated patients? Consider the fierce warnings issued by national medical organizations and state medical boards, threatening to strip any doctor who questions the safety of the COVID-19 vaccines of his license. If questioning vaccine safety can destroy your career and livelihood, would you create a permanent public record attaching your name and license number to a report doing precisely that?

Fatally Flawed Safety Reviews

Again, it is not spreading disinformation to take note of these figures, to ask what they mean, and to raise the possibility that the true extent of these events is underrepresented. But doesn't all this imply that the safety review process established by the FDA and pharmaceutical companies for the COVID-19 vaccines may be fatally flawed?

In a word, yes. From the revelations of a whistleblower about the "poor practices" and "data integrity" issues at a Pfizer subcontractor involved in the safety trials, to Pfizer's minimizing of catastrophic injuries as minor discomfort in the trial for the 12-15-year-old age group, to potential conflicts of interest on the FDA's vaccine advisory committees, there are plenty of reasons to be gravely concerned about the integrity of the safety review process for the COVID-19 vaccines.

Just as pressing are these questions: how did the process become so badly broken, and why have all the traditional independent stewards of the public interest, including the news media and academia, remained silent in the face of so many glaring failures? More than that, why have they been complicit in the censorship silencing anyone who raises these issues?

Questioning the competence and integrity of government bureaucracies like the FDA doesn't make someone a bad person or a spreader of disinformation. Government bureaucracies can be wrong, and historically the citizens of democracies have viewed it as not only their right but their duty to scrutinize public officials' decisions. Dissent is an integral part of the sacred compact between government and governed that underpins a free society, and Americans allow the current regime of censorship to continue at their extreme peril.

Dr. Harvey Risch is professor of epidemiology at the Yale School of Public Health, Yale School of Medicine, and Yale Cancer Center. He published the seminal paper on early treatment of high-risk COVID-19 outpatients in the American Journal of Epidemiology and gave testimony about early COVID-19 treatment before the U.S. Senate in November 2020. Dr. Robert W. Malone is a pioneer of mRNA technology and authored groundbreaking research on how RNA could be delivered into cells. Dr. Byram Bridle is an associate

professor of viral immunology at the University of Guelph in Canada, whose research team studies the body's immune response to viruses and develops vaccines to prevent infectious diseases and immunotherapies to treat cancers.

Harvey Risch, Robert W. Malone, and Byram Bridle

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This is Exhibit DD referred to in the Reply Affidavit of David Fisman sworn by David Fisman of the City of Toronto, in the Province of Ontario, before me on March 15, 2024, in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Guess What? Segregation of Unvaccinated People to Protect the 'Vaccinated' is NOT Supported by Modeling Practiced With Integrity

DR. BYRAM W. BRIDLE
DEC 14, 2023

It has been two years, four months, and twenty-two days (873 total days) since the administration of my employer, the University of Guelph, banned me from accessing my office and laboratory. I would like to have access to my work spaces. Segregation makes me feel less than human.

- B. Bridle -

April 25, 2022, is a day that will live on in infamy within the sphere of Canadian science. It is the day that the worst piece of trash that I have ever seen was published in the Canadian Medical Association Journal. This paper, which can serve no more useful purpose than wiping one's soiled butt crack, can be found here.

Astonishingly, despite massive pushback and calls for retraction, the article remains published. It was blatantly obvious to most critically thinking laypeople that the article was utter trash. It could not be disguised that the 'modeling' created in a failed attempt to justify a pre-determined conclusion was concocted in a way that defied logic. It also contradicted existing real-world data, which is supposed to be used to determine whether a theoretical model holds any validity. As such, the ongoing presence of this paper in the world of science is a roaring blight on the integrity of the authors, and the Canadian Medical Association, as well as the University of Toronto and Canadian Institutes of Health Research, which supported the publication of pure horror science fiction. The paper was used to wrongfully promote hatred against and segregation of critical thinkers, all while spitting in the faces of scientific reality and academic integrity.

I wrote an analysis of this hatred-promoting article, which can be found at this link. Like the song about the Grinch, I say "stink, stank, stunk"!

An entire book with keen insights has been written about this, which you can find here.

Importantly, just today, two scientists have just published a peer-reviewed scientific paper that did the mathematical modeling a proper way. You can find this hot-off-the-press article here.

Here is the full citation...

> Societal Segregation Based on Vaccination Status. *Cureus* 15(12): e50520. doi:10.7759/cureus.50520

And here is the overall conclusion...

> *"we cannot recommend that SIR* [susceptible-infectious-recovered] *modelling be used to motivate or justify segregation policies regarding viral respiratory diseases, in the present state of knowledge"*

I recall that the paper published back in 2022 was gobbled up by legacy media like pigs at a trough and spread throughout the globe like manure upon a field within hours of going online.

I suspect the same urgency in disseminating science will be applied by the legacy media to this just-released publication that corrects the glaring errors of the previous one. Or, might I be wrong on that note?!? Surely, the legacy media would be craving to undo the massive harm they wrongfully yet gleefully propagated in 2022, wouldn't they?

---

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Jake McGee Dec 14, 2023

Pathetic MDs here in the US, pulling aside their masks to gobble the commie pabulum out of fear and/or intellectual ignorance. Those of us who refused the jab were mocked and vilified; an MD "friend" openly tried to shame me into it. "Well, I'll visit you in the hospital."

Shocking to see so many who still buy the narrative. Fauci, et al, the creators of this evil, still held quite nicely unaccountable.

2024 promises to be a banner year. Love the Canada truckers!

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1 reply

Bobbie Cameron Dec 14, 2023

I for one sm so thankful for you , for standing for truth🙏 I am not complaining but I am super limited in funds , I would dearly love to support you in funds however that I cannot do at present but I can tell you how important you are to me & so many others . You are a warrior on the front line 🤜🥰❤️🙏 thank you & Gid bless you

LIKE (35) REPLY SHARE

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DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

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PROCEEDING COMMENCED AT TORONTO

REPLY AFFIDAVIT OF DAVID FISMAN

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Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

AFFIDAVIT OF DR. JASON KINDRACHUK

I, Dr. Jason Kindrachuk, of the City of Winnipeg, in the Province of Manitoba, MAKE OATH AND SAY:

1. I have been asked by counsel to the defendant, Dr. David Fisman, to provide an expert opinion on the claims expressed on the COVID-19 vaccine safety and efficacy by Dr. Byram Bridle in June 2021 as part of this litigation**.** I am a virologist, specializing in medical microbiology and infectious diseases, and, as such, have knowledge of the matters contained in this Affidavit. A copy of my CV is attached hereto as **Exhibit "A"**.

2. Attached hereto as **Exhibit "B"**, is a copy of my Expert Report dated March 15, 2024.

3. Attached hereto as **Exhibit "C"** is an executed copy of my Acknowledgment of Expert's Duty Form 53 dated March 15, 2024.

SWORN by Dr. Jason Kindrachuk in the City of Montreal, in the Province of Quebec, before me at the City of Toronto, in the Province of Ontario, on March 15, 2024 in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits
(or as may be)

KATHERINE R. COSTIN

DR. JASON KINDRACHUK

This is Exhibit "A" referred to in the Affidavit of Dr. Jason Kindrachuk in the City of Montreal, in the Province of Quebec, before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Jason Kindrachuk, PhD
Laboratory of Emerging & Re-emerging Viruses
Department of Medical Microbiology & Infectious Diseases
University of Manitoba
Winnipeg, MB, Canada
Tel: (204) 789-3807
Email: Jason.Kindrachuk@umanitoba.ca

EDUCATION

2002-2007 **Ph.D., Department of Biochemistry**
University of Saskatchewan, Saskatoon, SK, Canada
Supervisor: Dr. Scott Napper
Thesis Title: *Host and Pathogen Sensory Systems as Targets for Therapeutic Intervention*

1996-2001 **B.Sc. (Honors), Department of Biochemistry**
University of Saskatchewan, Saskatoon, SK, Canada

PROFESSIONAL EXPERIENCE

2023-current **Associate Professor**
Canada Research Chair
Laboratory of Emerging and Re-Emerging Viruses
Department of Medical Microbiology and Infectious Diseases
Manitoba Centre for Proteomics and Systems Biology
Department of Internal Medicine
University of Manitoba
Winnipeg, MB

2017-2023 **Assistant Professor**
Canada Research Chair
Laboratory of Emerging and Re-Emerging Viruses
Department of Medical Microbiology and Infectious Diseases
University of Manitoba
Winnipeg, MB

2014-2016 **Staff Scientist**
Critical Care Medicine Department
Clinical Center
National Institutes of Health, Bethesda, MD, USA

Sept 2014 **Scientific Lead – Field Diagnostics**
Ebola Virus Disease Outbreak Response Efforts
Centers for Disease Control/Department of Defense Joint Operations
Monrovia, Liberia

2013-2014 **Principal Research Scientist**
Battelle Memorial Institute
Integrated Research Facility

National Institutes of Allergy and Infectious Diseases
National Institutes of Health, Frederick, MD, USA

2009-2013 **Visiting Fellow**
Emerging Viral Pathogens Section
National Institutes of Allergy and Infectious Diseases
National Institutes of Health, Bethesda, MD, USA

2007-2009 **Postdoctoral Fellow**
Centre for Microbial Diseases and Immunity Research
Department of Microbiology and Immunology University of British Columbia, Vancouver, BC, Canada

SCIENTIFIC AFFILIATIONS & COMMITTEE ACTIVITIES

Affiliations

2023-present **Co-Chair** – Department of Medical Microbiology & Infectious Diseases Graduate Studies Committee

2021-present **Scientific Advisory Board** – Canadian Consortium of Academic Biosafety Level 3 Laboratories (CCABL3)

2021-present **Co-Lead** – CoVaRR-Net (Pillar 2: Host-Pathogen Interactions)

2020-present **Visiting Scientist** – Vaccine and Infectious Disease Organization-International Vaccine Centre

2020-present **Science Contributor** – Forbes Media, LLC

2020-present **Volunteer, Infection Control Lead** – Heart to Heart International COVID-19 Preparedness and Response Efforts

2019-present **Visiting Researcher** – The International Center for Medical Research in Franceville, Gabon (CIRMF)

2018-present **Visiting Scientist** – Public Health Agency of Canada, Special Pathogens Section

2018-present **Visiting Scientist** – Canadian Food Inspection Agency, Special Pathogens Unit

2017-present **Investigator** – Children's Health Research Institute of Manitoba

Expert Committees

2023-current **Member** – Advisory Committee on Human Pathogens and Toxins

2023 **Member** – Pandemic Instrument Partner and Stakeholder Engagement Forum

2023 **Delegate** – Canada's Science Meets Parliament Program

2023 **CEZD Representative** to the CAHSS Steering committee

2022 **Panelist** – Towards the development of a global CORE protocol for evaluation of treatments for hMPXV Leveraging the Congolese Experience. INRB and the ANRS |Emerging Infectious Diseases/INSERM] in collaboration with WHO

2022-current **Member** – World Health Organization Guideline Development Group (GDG): Clinical management and infection prevention control guidance for monkeypox

2022-current **Member** – World Health Organization (WHO) Health Emergencies (WHE) Infection Prevention and Control (IPC) Hub - MPOX Inactivation

2020	**Panel Member** – CIHR Institute of Infection and Immunity Consultation on Variant Strains of SARS-CoV-2
2020-current	**Member** – World Health Organization COVID-19 Solidarity Serology Study Group
2020-current	**Member** – World Health Organization Working Group on Assays & Animal Models - Priority Pathogens
2019-current	**Member** – Community for Emerging and Zoonotic Diseases, Canadian Network for Public Health Intelligence (CNPHI)
2019	**Member** – CIHR Strategic Planning Meeting
2018-2022	**Director** – Canadian Society for Virology Executive Council
2018-current	**Member** – CIHR College of Reviewers
2018	**Co-Chair** – Emerging Viral Diseases and Global Preparedness Symposium
2018	**Organizing Committee Member** – Canada's Role in Global Public Health Conference
2012-2013	**Scientific Advisor** – World Health Organization Advisory Committee on Variola Virus Research (ACVVR)

Review Panels

2022-current	**Review Panel** – Canadian Institutes of Health Research Project Grant Competition
2019-current	**Review Panel** – New Frontiers in Research Fund Exploration Grant
2017-2020	**Review Panel** – American Association for the Advancement of Science (AAAS) Research Competitiveness Program
2017-current	**Reviewer** – Manitoba Poster Competition of the Canadian Student Health Research Forum
2017-current	**Review Panel** – Research Manitoba Studentship Competition
2016	**External Reviewer** – National Science Centre, Poland (Narodowe Centrum Nauki –NCN) PRELUDIUM Funding scheme
2014	**Reviewer** – Alberta Livestock and Meat Agency (ALMA)

AWARDS & HONOURS

2022	University of Manitoba Merit Award - Combination of Teaching, Service and Research
2021-2026	Tier 2 Canada Research Chair Award (renewal)
2021	Ken Hughes Young Investigator's Award in Medical Research
2021	Campbell Outreach Award
2018	Department of Medical Microbiology Faculty Educator Award
2018	National Institutes of Allergy and Infectious Diseases Merit Award
2017-2021	Tier 2 Canada Research Chair Award
2015	National Institutes of Health Director's Award

2015	National Institutes of Health Clinical Center Summer Internship Program Best Mentor Award
2013	University of Maryland Integrated Life Sciences Honors College Mentor Award
2010-2013	National Institutes of Health Visiting Fellow Intramural Research Training Award

SCIENTIFIC JOURNAL ADVISORY BOARDS

2019-present **Guest Editor** – Viruses (Pathogenesis of Emerging Viruses Special Issue)

2019-present **Associate Editor** – Viruses

2019-present **Associate Editor** – Frontiers in Microbiology

2016-present **Associate Editor** – BMC Infectious Diseases

2014-present **Associated Review Editor** – Frontiers in Veterinary Science

PROFESSIONAL ASSOCIATIONS & MEMBERSHIPS

2019-present	**Member** – Infectious Diseases Society of America
2017-present	**Member** – Canadian Society for Virology
2017-present	**Member** – Canadian Society of Microbiologists
2014-present	**Member** – American Society for Microbiology
2014-present	**Member** – American Society for Virology

PUBLICATIONS

1. Savinkina, A., **Kindrachuk, J.**, Bogoch, I.I., Rimoin, A.W., Hoff, N., Shaw, S.Y., Mbala-Kengebeni, P., and Gonsalves, G.S. Modeling vaccination approaches for mpox containment and mitigation in the Democratic Republic of the Congo. *Lancet*. [In Submission]
2. Schindell, B.G., Kangbai, J.B., Fredborg, B., Kowalec, K., Shaw, S.Y., and **Kindrachuk, J.** The state of mental health amongst Ebola virus disease survivors through a cross-sectional study in Sierra Leone. *BMJ Mental Health*. [In Submission]
3. Webb, A.L., Schindell, B., Soule, G., Siddik, A.B., Abrenica, B., Memon, H., Su, R., Safronetz, D. and **Kindrachuk, J¶.** Sertoli cells remain viable and inhibit viral replication during Ebola virus infection. *NPJ Viruses*. [Accepted]
4. Connelly, M.S., Warner, S., Swerczek, J., **Kindrachuk, J.**, Vannella, K.M., Ramos-Benitez, M.J., Strich, J.R., Sun, J., Dougherty, E., Danner, R., Moore, I.M., Herbert, R., and Chertow, D.S. A Model of prolonged sedation and supportive care in rhesus macaques for the investigation of human critical illness. *Lab Animal*. [In Revision]
5. Stein, D., **Kindrachuk, J.**, McKinnon, L., Reimer, J., Bullard, J., Van Caesseele, P., and Shaw, S. The descriptive epidemiology of pre-Omicron SARS-CoV-2 breakthrough infections and severe outcomes in Manitoba. *Front. Epidemiol*. [Accepted]
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BOOK CHAPTERS

1. Chertow, D.S. and **Kindrachuk, J.** (2019) Respiratory Viruses: Influenza, Measles, SARS, MERS, and Smallpox. In: Highly Infectious Diseases in Critical Care: A Comprehensive Clinical Guide. Springer New York.

2. Falcinelli, S.D., Ciric, J., **Kindrachuk, J.** (2018) Variola Virus: Clinical, Molecular and Bioterrorism Perspectives. In: Defense Against Biological Attacks. Springer New York.

3. Jahrling, P.B., Goff, A.J., Johnston, S.C., **Kindrachuk, J.**, Lin, K.L., Huggins, J.W., Ibrahim, S.,

Lawler, J.V., Martin, J.W. (2016) Chapter 11: Smallpox and Related Orthopoxviruses. In: Textbooks of Military Medicine: Medical Aspects of Biological Warfare.

4. **Kindrachuk, J.**, Kuhn, J.H. and Jahrling, P.B. (2015) The role of viral protein phosphorylationduring filovirus infection. In: Global Virology. Springer New York.
5. **Kindrachuk, J.** and Napper, S. (2013) Sample Preparation and Profiling: Probing the kinome for biomarkers and therapeutic targets: peptide arrays for global phosphorylation-mediated signal transduction. In: Comprehensive Biomarker Discover and Validation for Clinical Application. Royal Society of Chemistry.
6. Petersen, P.D., **Kindrachuk, J.**, Jenssen, H. (2012) Immune modulation for treatment of herpes simplex virus. In: Herpes Simplex Virus: Prevention, Recognition and Management. In: Advances in Medicine and Biology. Nova Science Publishers.
7. **Kindrachuk, J.**, Nijnik, A. and Hancock, R.E.W. (2009) Ch. 5.07 Host Defense Peptides: Bridging Antimicrobial and Immunomodulatory Activities. In: Mander, L., and Liu, H.W. Comprehensive Natural Products Chemistry II. Oxford: Elsevier.

NON-PEER REVIEWED PUBLICATIONS

1. COVID-19 Delta variant in Canada: FAQ on origins, hotspots and vaccine protection. *The Conversation*. https://theconversation.com/covid-19-delta-variant-in-canada-faq-on-origins-hotspots-and-vaccine-protection-162653
2. Why Early Wins Over Covid-19 Do Not Mean Victory: Canada's Cautionary Tale. *Forbes.* https://www.forbes.com/sites/coronavirusfrontlines/2020/11/04/why-early-wins-over-covid-19-do- not-mean-victory-canadas-cautionary-tale/?sh=740b94722041
3. The Realities of Biomedical Research During a Pandemic. *Forbes.* https://www.forbes.com/sites/coronavirusfrontlines/2020/09/17/a-virologist-explains-the-realities- of-biomedical-research-during-a-pandemic/#62d6a66111a2
4. A Virologist Explains Why It Is Unlikely COVID-19 Escaped from A Lab. *Forbes*. https://www.forbes.com/sites/coronavirusfrontlines/2020/04/17/a-virologist-explains-why-it-is-unlikely-covid-19-escaped-from-a-lab/#58bd5fb63042
5. How The Coronavirus Pandemic Has Impacted International Research Programs: A Personal Perspective. *Forbes.* https://www.forbes.com/sites/coronavirusfrontlines/2020/06/06/how-the-coronavirus-pandemic- has-impacted-international-research-programs-a-personal-perspective/#b10049750bbf
6. Repurposing Drugs Is Key to Fighting the Coronavirus Pandemic, This Virologist Explains. *Forbes*. https://www.forbes.com/sites/coronavirusfrontlines/2020/05/08/repurposing-drugs-is-key-to- fighting-the-coronavirus-pandemic-this-virologist-explains/#5c9efd1217ce
7. No Mercy for the Coronas. *La Liberté*. https://www.lalibertesciencesmagjunior.ca/
8. The Value of Social Media Now. *New York Times*. https://www.nytimes.com/2020/03/27/opinion/letters/iran-sanctions.html
9. How social media is changing research and reactions to coronavirus outbreak. *The Conversation*. http://theconversation.com/how-social-media-is-changing-research-and-reactions-to-coronavirus-outbreak-130748
10. Ebola survivors can pass on the virus: we're trying to understand what role sex plays. *The Conversation Africa*. https://theconversation.com/ebola-survivors-can-pass-on-the-virus-were-trying-to-understand- what-

role-sex-plays-124015

REPORTS

1. Clinical management and infection prevention control guidance for monkeypox: interim guidance. World Health Organization Guideline Development Group (GDG); 2022 June. https://www.who.int/publications/i/item/WHO-MPX-Clinical-and-IPC-2022.1
2. CIHR-PHAC-CADTH – Best Brains Exchange – Transmission Routes for COVID-19: Implicationsfor Public Health. Canadian Institutes of Health Research (CIHR); 2020 October. https://cihr-irsc.gc.ca/e/52238.html
3. Heating, Ventilation and Air Conditioning Systems in Public Spaces. Ottawa: Canadian Agency for Drugs and Technologies in Health (CADTH); 2020 June. (CADTH technology review).
4. Public statement for collaboration on COVID-19 vaccine development. World Health Organization.13 April 2020. https://www.who.int/news-room/detail/13-04-2020-public-statement-for- collaboration-on-covid-19-vaccine-development
5. WHO Advisory Committee on Variola Virus Research, 15th Meeting. Report of the Fourteenth Meeting, Geneva, Switzerland, 24-24 September 2013. WHO/HSE/PED/CED/2013.2
6. WHO Advisory Committee on Variola Virus Research, 14th Meeting. Report of the Fourteenth Meeting, Geneva, Switzerland, 16-17 October 2012. WHO/HSE/PED/CED/2013.1

FUNDING

1. Impact of host responses on mpox pathogenesis and tecovirimat efficacy in the Collaborative Cross mouse model of genetic diversity
   Funding Sources:
   Team Grant: Building capacity in interdisciplinary research on mpox (monkeypox) and other (re)emerging zoonotic threats
   Total Funding – 500,000 (Canadian dollar)
   Co-Principal Investigator
2. Investigation of Mpox virus spillover and spillback at the human-animal interface in the Democratic Republic of Congo
   Funding Sources:
   Team Grant: Building capacity in interdisciplinary research on
   mpox (monkeypox) and other (re)emerging zoonotic threats
   Total Funding – 500,000 (Canadian dollar)
   Co-Investigator
3. The Pandemic Readiness Approach & Innovative Response Initiative Ecosystem (PRAIRIE) Hub
   Funding Sources:
   Canadian Biomedical Research Fund
   Total Funding – 2,000,000 (Canadian dollar)
   Co-Investigator
4. Characterization of monkeypox virus circulation and transmission from wildlife to humans in Africa and identification of wildlife species at elevated risk for infection in Canada.
   Funding Sources:
   2021-2026 Canadian Institutes of Health Research Project Grant
   Total Funding – 750,000 (Canadian dollar)
   Principal Investigator
5. A prospective and retrospective multi-center, cohort study for clinical, virologic and immunologic characterization of monkeypox virus clade IIb by the International Monkeypox Response Consortium

(IMREC).
Funding Sources:
Team Grant: Monkeypox Rapid Research Response (Canadian Institutes of Health Research & International Development Research Centre)
Total Funding – $2,840,500 (Canadian dollar)
Principal Investigator

6. Tier 2 Canada Research Chair in the molecular pathogenesis of emerging and re-emerging viruses.
Funding Sources:
2021-2026 Canada Research Chairs Program
Total Funding – 500,000 (Canadian dollar)
Principal Investigator

7. Tier 2 Canada Research Chair in the molecular pathogenesis of emerging and re-emerging viruses.
Funding Sources:
2017-2022 Canada Research Chairs Program
Total Funding – 500,000 (Canadian dollar)
Principal Investigator

8. The landscape of risk: examining the correlates of inequitable COVID-19 infection and vaccination rates in Manitoba using population-based laboratory and administrative healthcare data.
Funding Sources:
CIHR Emerging COVID Research Gaps and Priorities
Total Funding - $239,942
Co-Investigator

9. Identification of the molecular determinants underlying asymptomatic Ebola virus testicularinfections and long-term effects on reproductive health
Funding Sources:
2021-2026 Canadian Institutes of Health Research Project GrantTotal Funding – 726,500 (Canadian dollar)
Principal Investigator

10. Cryo-electron microscopy (cryo-EM) to guide rapid development of novel therapeutic strategiesand improved diagnostics for COVID-19
Funding Sources:
Canadian Foundation for Innovation
Total Funding – 950,000 (Canadian dollar)
Co-Applicant

11. Animal models for SARS-CoV-2: vaccines and immune enhancement
Funding Sources:
2020-2022 Canadian Institutes of Health 2019 Novel Coronavirus (COVID-19) Rapid Research Funding
Total Funding – 999,793 (Canadian dollar)
Co-Applicant

12. Scalable, Customizable, Digital Health Communication Materials to Help Canada Address the COVID19 Pandemic
Funding Sources:
2020-2021 Canadian Institutes of Health COVID-19 Rapid Research - Social Policy and Public Health Responses
Total Funding – 311,296 (Canadian dollar)
Co-Applicant

13. Broad Spectrum CoV Therapeutic; rhACE2 Immunoadhesin to treat COVID19
Funding Sources:

2020-2021 MITACS Accelerate
Total Funding – 90,000 (Canadian dollar)
Principal Applicant

14. Prairie Infectious Immunology Network 2020
Funding Sources:

2020-2021 Canadian Institutes of Health Research Planning and Dissemination GrantTotal Funding – 10,000 (Canadian dollar)
Co-Principal Investigator

15. Characterization of the molecular pathogenesis of severe influenza and influenza-bacterialinfections at the alveolar-capillary barrier
Funding Sources:
2018-2020 Research Manitoba New Investigator Operating GrantTotal Funding – 130,000 (Canadian dollar)
Principal Investigator

16. Investigation of kinase-mediated cell signaling pathway modulation at the vector pathogen-livestock interface in vector-borne livestock diseases
Funding Sources:
2018/4 - 2023/3 Natural Sciences and Engineering Research Council of Canada(NSERC) Discovery Grant
Total Funding - 165,000 (Canadian dollar)
Principal Investigator

17. Establishment of a high-throughput molecular dynamics facility
Co-applicant: Denice Bay
Funding Sources:
2017/11 - 2022/11 Canada Foundation for Innovation (CFI) John R. Evans Leaders FundTotal Funding - 609,191 (Canadian dollar)
Principal Investigator

18. Deciphering the bat kinome by immunometabolic peptide kinome arrays: critical insights for emerging viral diseases
Funding Sources:
2018/7 - 2019/6 University of Manitoba Dr. Paul H. T. Thorlakson Foundation FundTotal Funding - 30,000 (Canadian dollar)
Principal Investigator

19. Characterizing the molecular mechanisms of Ebola virus persistence at the blood-testis barrier
Funding Sources:
2018/8 - 2019/4 University of Manitoba Tri-Agency Bridge FundingTotal Funding - 60,000 (Canadian dollar)
Principal Investigator

20. Characterizing the molecular mechanisms of Ebola virus persistence in a 3D co-culture model ofthe blood-testis barrier
Funding Sources:
2018/3 - 2019/3 Manitoba Medical Service Foundation (MMSF) Operating GrantTotal Funding - 19,219 (Canadian dollar)
Principal Investigator

21. Capacity Building Projects in an Institution of Higher Learning in the Developing World
Funding Sources:
2018/1 - 2019/10 University of Manitoba International Program and Partnership SeedTotal Funding – 5,000 (Canadian dollar)

Principal Investigator

INVITED PRESENTATIONS

1. Investigating emerging zoonotic virus circulation and spillover at the human-wildlife interface in diverse global settings – increasing preparedness and response capacities. **Canadian Biosafety Symposium Program.** 2023
2. How did I get here?! Navigating shifting career trajectories within and outside of the Hot Zone. **Feature Speaker Connection.** Biochemistry, Microbiology & Immunology Student Association. University of Saskatchewan. 2023
3. International Mpox Response Consortium (IMREC): A Prospective and Retrospective Multi-center, Cohort Study For Surveillance, Clinical Characterization And Determination Of Relative Vaccine Effectiveness For Monkeypox Virus. **XXIV International Poxvirus, Asfavirus, and Iridovirus Conference.** Dusseldorf, Germany. 2023
4. Biomedical research trajectories: what is the R2 of time versus research independence? **MMSF Showcase Lecture Speaker. Manitoba Student Health Research Forum.** 2023
5. The Best Offense is a Good Defense: emerging zoonotic virus preparedness in the lab and in the field. What Could Cause the Next Pandemic? **The Scientist.** 2023
6. What Virus Goes There? Tracking emerging zoonotic virus circulation in the lab and in the field. Department of Medical Microbiology & Immunology. **University of Alberta.** 2023
7. What Virus Goes There? Tracking emerging zoonotic virus circulation in the lab and in the field. Plenary seminar. **Queen's University.** 2022
8. Panelist - Towards the development of a global CORE protocol for evaluation of treatments for hMPXV Leveraging the Congolese Experience. **INRB and the ANRS |Emerging Infectious Diseases/INSERM] in collaboration with WHO**. Virtual. 2022
9. Panel Member - A COVID 19 VACCINE WEBINAR. **The Canadian Foundation for Infectious Diseases. Virtual. 2022**
10. Panel Member - Science communication, media, and knowledge mobilization. Renascent 2022 - **hosted by CIHR-ICRH, The Canadian Lung Association, the Canadian Respiratory Research Network, and the Canadian Thoracic Society**. Virtual. 2022
11. Looking back…to see more clearly? **ACCORD Webinar Series. Children's Hospital Research Institute of Manitoba.** Virtual. 2022
12. Has it really been two years already? A candid discussion on emerging virus spillovers and persistence. **Institute of Cardiovascular Sciences (ICS). St. Boniface Research Centre.** Virtual. 2022
13. Keynote seminar: What virus goes there? Emerging virus research from the field to the lab. **2021 Canadian Biosafety Virtual Symposium**
14. What virus goes there? Emerging virus research from the field to the lab. **Tulane University**. Virtual. 2021
15. Universal coronavirus vaccines: How close are we? **American Thoracic Society AnnualMeeting**. Virtual. 2021
16. What virus goes there? Emerging virus research from the field to the lab. Molecular Biology & Biochemistry Department. **Simon Fraser University**. Virtual. 2021
17. Covid-19 and vaccine safety. Bisons Athletics. **University of Manitoba**. Virtual. 2021

18. Covid Q&A Part II with Raman Dhaliwal and Dr. Jason Kindrachuk. **University of Manitoba**.Virtual. 2021
19. Current perspectives on Covid-19. **British Columbia Dental Association**. Virtual. 2021
20. Covid-19: Current state of knowledge. **Wastewater Epidemiology Group**. Public Health Agencyof Canada. Virtual. 2021
21. Covid-19: Current state of knowledge. **Covid-19 Genome Sequencing Group**. Public Health Agency of Canada. Virtual. 2021
22. Vaccines & therapeutics for COVID-19. **Café Scientifique**. Virtual. 2021
23. Balancing science and disinformation during Covid-19. Stem Skills for the 21st Century. **Bioscience Association of Manitoba**. Virtual. 2021
24. Eleven Covid months equals one decade - Emerging virus research during a pandemic. **Quebec Centre for Advanced Materials (QCAM)**. Virtual. 2020
25. Emerging Virus Research in the Time of Covid. Global Health Seminar Series. **Tel AvivUniversity**. Virtual. 2020
26. COVID-19 Transmission: Current state of virology knowledge. **Community-based aerosol transmission of Covid-19 and HVAC systems**. Canadian Agency for Drugs and Technologies in Health (CADTH). Virtual. 2020
27. Transmission Routes for COVID-19: Implications for Public Health. **CIHR-PHAC-CADTH – Best Brains Exchange**. Canadian Institutes of Health Research (CIHR). Virtual. 2020
28. Heating, Ventilation and Air Conditioning Systems in Public Spaces. Ottawa: **Canadian Agencyfor Drugs and Technologies in Health (CADTH)**. Virtual. 2020
29. 2020 Fall Member Forum: What's Next? The Aftermath of the COVID-19 Crisis. **Western Transportation Advisory Council (WESTAC)**. Virtual. 2020
30. Basic, Translational and Public Health Research During a Novel Pandemic. **School of Public Health, University of Saskatchewan**. Virtual. 2020
31. Characterizing tissue-barrier specific pathogenesis of epidemic and pandemic emerging viruses. **Infectious Disease, Microbiome, and Public Health Conference**. Virtual. 2020
32. COVID-19: Early Assessments of the First Coronavirus Pandemic. **Value Partners AnnualGeneral Meeting**. Virtual. 2020
33. COVID-19 and infection, prevention and control. **Canadian Dental Association**. Virtual. 2020
34. COVID-19: The Emergence and Spread of a Pandemic in the Age of Social Media. **UM Learningfor Life Program**. University of Manitoba. Virtual. 2020
35. COVID-19: Monitoring the Emergence and Pandemic Spread of SARS-CoV-2 in Real Time. **International Life Sciences Institute – North America**. Virtual. 2020
36. Characterizing Emerging Virus Circulation and Spillovers in West and Central Africa. **Society of Clinical Research Associates**. Winnipeg, Canada. 2020
37. The Real Hot Zone: Studying Emerging Virus Circulation and Spillover in the Lab and the Field. **Department of Microbiology, Immunology & Infectious Diseases, University of Calgary**. 2020
38. COVID-19: An Emerging Public Health and Economic Crisis. **Manitoba Young Presidents Organization**. Winnipeg, Canada. 2020
39. Characterizing emerging virus circulation and spillovers in West and Central Africa. **Annual University of Nairobi HIV/AIDS Collaborative Conference**. Nairobi, Kenya. 2020

40. Identifying the molecular determinants underlying Ebola virus persistence in incidental and reservoir hosts. **KAVI Institute for Clinical Research**. University of Nairobi, Nairobi, Kenya. 2020
41. Characterizing the molecular determinants underlying severe Ebola virus disease and post-recovery persistence. **International Infection, Immunity and Inflammation Conference (I4C)**, Vancouver, Canada. 2019
42. Investigating the molecular pathogenesis of emerging and re-emerging viruses at the interface of basic and clinical research. **Manitoba Chemistry Symposium**, Winnipeg, Canada. 2018
43. Navigating the Storm: merging basic research with clinical information for (re)emerging infectious diseases. **Canadian Society of Microbiologists Annual Meeting**, Winnipeg, Canada. 2018
44. Are We Ready for the Next Pandemic? Reflections from the laboratory and the field**. CPDMedicine Program: Trends & Challenges in Virology**, Winnipeg, Canada. 2017
45. Investigating Interactions between the Host and High-Consequence Pathogens with Systems Kinomics. **University of Delaware Graduate Student Seminar Series**. University of Delaware,DE. 2015.
46. Characterizing High-Consequence Pathogens through Systems Kinome Analysis. **NICBR Exploring Careers in a Scientific Environment Symposium (NECSES)**. Fort Detrick, Frederick, MD, 2014.
47. Species-Specific Kinome Analysis for the Investigation of the Molecular Pathogenesis of High-Consequence Pathogen and Identification of Novel Therapeutic Targets. **American Society of Virology**. Fort Collins, CO, 2014.
48. Temporal kinome analysis demonstrates Ebola virus selectively modulates transforming growth factor β signaling. **6th International Symposium on Filoviruses**. Galveston, TX, 2014.
49. Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention. **15th Meeting of the WHO Advisory Committee on Variola Virus Research.**Geneva, Switzerland, 2013.
50. Systems kinome analysis of differential host responses to variola virus and monkeypox virus. **US Delegation to WHO.** Eisenhower Office Building, Washington, DC, 2013.
51. Ebola virus selectively modulates transforming growth factor-β signaling as demonstrated by temporal kinome analysis. **American Society of Virology.** Pennsylvania State University, PA,2013.
52. Investigating high-consequence viral pathogenesis under (negative) pressure. **Vaccine and Infectious Disease Organization (VIDO).** Saskatoon, SK, 2012.
53. Use of live variola virus in systems kinomics for identification of host targets for therapeutic intervention. **14th Meeting of the WHO Advisory Committee on Variola Virus Research.**Geneva, Switzerland, 2012.
54. Temporal kinome analysis of Ebola virus molecular pathogenesis. **Centers for Disease Control (CDC): Special Pathogens Branch.** Atlanta, GA, 2012.
55. Temporal systems kinomics analysis of host cell responses to Ebola virus. **Keystone Symposium: Cell Biology of Virus Entry, Replication and Pathogenesis (X7).** Whistler, BC,2012.
56. Kinome analysis reveals differential host cell responses to West African and Congo basin monkeypox virus. **Gordon Research Conference – Chemical and Biological TerrorismDefense.** Ventura, CA, 2011.

EXPERT WITNESS CONTRIBUTIONS

1. 2021-2022 – Government of Canada

2. 2021-2022 – Alberta Government
3. 2020-2022 – Manitoba Government

SELECTED MEDIA LINKS

News Articles

1. Mpox outbreak in the Democratic *Voice of America. Africa News Tonight*. https://www.voaafrica.com/a/7366661.html
2. Mpox surge in Congo raises concerns world will ignore warnings again. *The Washington Post*. Mpox surge in Congo raises fears world will ignore warnings again - The Washington Post
3. Amid Congo's deadliest mpox outbreak, a new worry: virus has become sexually transmissible. *Science*. Amid Congo's deadliest mpox outbreak, a new worry: virus has become sexually transmissible | Science | AAAS
4. Tokyo Olympics: Can vaccines save the Games? *CNN*. https://www.cnn.com/2021/01/29/sport/tokyo-olympics-vaccines-cmd-spt-intl/index.html
5. The Health 202: The pandemic intensified the tech censorship debate. *The Washington Post*. https://www.washingtonpost.com/politics/2021/06/07/health-202-pandemic-intensified-tech-censorship-debate/
6. Yes, vaccines block most transmission of COVID-19. *National Geographic*. https://www.nationalgeographic.com/science/article/yes-vaccines-block-most-transmission-of- covid-19
7. Inside the global race against COVID-19 mutations. *Maclean's*. https://www.macleans.ca/society/science/covid-variants-vaccination-race/
8. Canada could see COVID resurgence; only 4 percent of population have had both shots. *Newsweek*. https://www.newsweek.com/canada-covid-resurgence-1594280
9. Ramping up COVID-19 vaccine production is harder than it seems. *Popular Science*. https://www.popsci.com/story/health/mrna-covid-vaccine-ramp-up-production/
10. What's important to know about the new COVID-19 variants? *CMAJ.* https://www.cmaj.ca/content/193/4/E141
11. Bombshell analysis traces new Ebola outbreak to survivor of West Africa crisis. STAT News. https://www.statnews.com/2021/03/12/bombshell-analysis-traces-new-ebola-outbreak-to-survivor- of-west-africa-crisis/
12. Congo working to stop new Ebola outbreak in country's east. *Associated Press*. https://abcnews.go.com/Health/wireStory/congo-working-stop-ebola-outbreak-countrys-east-75745874
13. Ramping up COVID-19 vaccine production is harder than it seems. *Popular Science*. https://www.popsci.com/story/health/mrna-covid-vaccine-ramp-up-production/
14. Tokyo Games chief expects decision by March on allowing spectators. *Reuters*. https://www.reuters.com/article/olympics-2020-spectators-int-idUSKBN29H1DP
15. What scientists still want to know about the new coronavirus variant in the U.K. *NBC News.* https://www.nbcnews.com/health/health-news/what-scientists-still-want-know-about-new-coronavirus-variant-u-n1252122
16. Researchers propose process to detect and contain emerging diseases. *Science Daily.* https://www.sciencedaily.com/releases/2020/12/201218152727.htm
17. Canada's coronavirus performance hasn't been perfect. But it's done far better than the U.S. *Washington Post*. https://www.washingtonpost.com/world/the_americas/coronavirus-canada-united-states/2020/07/14/0686330a-c14c-11ea-b4f6-cb39cd8940fb_story.html

18. Canada's Coronavirus Outbreak Slows as Cases Top 50,000, but Long Fight Looms. *NewYork Times*. https://www.nytimes.com/reuters/2020/04/29/world/americas/29reuters-health-coronavirus- canada-cases.html
19. Olympics: Organisers must be flexible if coronavirus vaccine not ready in time, experts say. *Reuters*. https://www.reuters.com/article/us-health-coronavirus-olympics-vaccine/olympics-organisers-must-be-flexible-if-coronavirus-vaccine-not-ready-in-time-experts-say-idUSKBN2220OU
20. Higher flu vaccination rates could help expose new viruses like Covid-19 earlier, expert says. *The Guardian*. https://www.theguardian.com/world/2020/apr/10/higher-flu-vaccination-rates-could-help-expose- new-viruses-like-covid-19-earlier-expert-says

Radio, Podcast and Television Interviews

Recurring Appearances

1. Weekly guest – *CTV News Channel*
2. Weekly radio guest – COVID-19 updates. *Charles Adler Tonight.*
3. Weekly radio guest – COVID-19 updates. *Sunday Night Health Show* (with Maureen McGrath).
4. Weekly radio guest – *The News with Richard Cloutier and Julie Buckingham*.
5. Recurring guest – Ask an Expert. *Reuters*
6. Recurring guest – *Ottawa at Work with Leslie Roberts. 580 CFRA*
7. Recurring guest – *Jody Vance*. *CKNW*
8. Recurring guest – *Lynda Steele Show*. *CKNW*
9. Recurring guest – *CBC News Network*
10. Recurring radio guest – Hal Anderson. *680 CJOB*
11. Recurring guest – COVID-19 updates. *Leading Britain's Conversation*
12. Recurring guest – COVID-19 safety restrictions and economic impacts. *Bloomberg News Network*.
13. Recurring guest – COVID-19 updates. *Viewpoints with Todd Vanderhayden*

Individual Appearances

14. The Covid Cruise. *The Nature of Things* https://www.cbc.ca/natureofthings/episodes/the-covid-cruise
15. Covid-19 vaccine hesitancy. *CNN Newsroom* (with John Avlon) http://www.cnn.com/TRANSCRIPTS/2104/08/cnr.18.html
16. COVID-19 Chapter 15: Disease, Take 2. *This Podcast Will Kill You*. https://podcasts.apple.com/gr/podcast/covid-19-chapter-15-disease-take-2/id1299915173?i=1000514995071
17. Getting to the truth on vaccines with Dr. Jason Kindrachuk – Infectious disease expert at the University of Manitoba. *No Nonsense with Pamela Wallin*. http://pamelawallin.com/getting-to-the-truth-on-vaccines-with-dr-jason-kindrachuk-infectious- disease-expert-at-the-university-of-manitoba/
18. Canada needs to be better prepared for the next pandemic, which can be very soon: Virologist. *Bloomberg News Network*. https://www.bnnbloomberg.ca/canada/video/canada-needs-to-be-better-prepared-for-the-next-pandemic-which-can-be-very-soon-virologist~2142993

PATENT SUBMISSIONS

1. Small Cationic Anti-biofilm and IDR Peptides.
   United States. PCT/US2014/052993. 2014/08/27.
   Patent Status: Pending

2. Combination adjuvant formulation.
   United States. US9408908 B2. 2013/02/15.
   Patent Status: Granted/Issued
   Year Issued: 2016

3. Immunomodulatory compositions and methods for treating disease with modified host defense peptides.
   United States. US9102754 B2. 2008/06/27.
   Patent Status: Granted/Issued
   Year Issued: 2015

This is Exhibit "B" referred to in the Affidavit of Dr. Jason Kindrachuk in the City of Montreal, in the Province of Quebec, before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

March 15, 2024

To whom it may concern:

I have been asked by counsel to the defendant, Dr. David Fisman to review the statements he made on the May 27, 2021 AM640 podcast "On Point with Alex Pierson". I have been asked to consider whether Dr. Bridle's statements at the time were data-based and accurate based on the information on COVID-19 vaccinations at the time.

In forming my opinion I have been provided with the following material:

1. Statement of Claim issued December 19, 2022;
2. Motion Record of the Defendant, Dr. Fisman, dated June 30, 2023;
3. Supplementary Motion Record of the Defendant, Dr. Fisman, dated July 21, 2023;
4. Responding Motion Record of the Plaintiff, Dr. Bridle, dated December 15, 2023; and
5. Transcript of the May 31, 2021 AM640 podcast "On Point with Alex Pierson".

1. Education and Professional Associations

I am a Tenured Associate Professor and Canada Research Chair in emerging viruses in the Department of Medical Microbiology & Infectious Diseases, University of Manitoba. My field of expertise is the investigation of emerging viruses, the infections they cause and their impact on global health. In particular, I have been actively involved in emerging virus research since 2009 with a focus on those viruses that are considered global health threats. These have included ebolaviruses, influenza viruses and coronaviruses. I have worked extensively within high containment laboratories including hundreds of hours in both containment level 3 and containment level 4 laboratories. My research activities include engagement with multiple international partners across sub-Saharan Africa including Democratic Republic of the Congo, Cameroon, Rwanda, Sierra Leone, and Kenya. Notably, this includes as Director of the Internatinoal Mpox Response Consortium, jointly funded by the Canadian Institutes of Health Research and the International Development Research Centre. I am the inaugural Director of the containment level 3 laboratory that is currently under renovation at the University of Manitoba. I serve as co-Director of Pillar 2 within the Coronavirus Variants Rapid Response Network (CoVaRR-Net), which was created to assist in the federal government's overall strategy to address the potential threat of emerging SARS-CoV-2 variants. I am also a selected member of the federal Advisory Committee on Human Pathogens & Toxins. I have served as an elected member (Director) of the Canadian Society for Virology Executive Committee for multiple terms. Internationally, I serve as a member of multiple World Health Organization (WHO)

committees including the WHO Guideline Development Group (GDG): Clinical management and infection prevention control guidance for monkeypox, the WHO Health Emergencies (WHE) Infection Prevention and Control (IPC) Hub - MPOX Inactivation, the WHO COVID-19 Solidarity Serology Study Group, and the WHO Working Group on Assays & Animal Models - Priority Pathogens. I previously served as an invited Advisory Committee on Variola Virus Research on multiple occasions.

My experience in outbreak response is as follows. In 2014 I helped lead diagnostic support efforts in the field for the Centers for Disease Control/Department of Defense joint operations in Monrovia, Liberia, during the West African Ebola virus disease epidemic, I am also a current volunteer with Heart to Heart International, a global humanitarian organization that focuses on improving public health and responding to the victims of disaster worldwide. In 2020, I provided infection prevention and control expertise in support of their COVID-19 response efforts virtually for the Marshall Islands and for small and medium-sized organizations in the US. Currently, I lead multiple mpox (formerly monkeypox) outbreak response activities in Democratic Republic of the Congo and in Cameroon. Our activities include ongoing serosurveillance studies among populations at increased risk for infection across the Canadian prairies.

My education history is as follows. I completed my undergraduate and graduate training at the University of Saskatchewan with a BSc Honors degree in 2002 and a PhD in 2007 in the Department of Biochemistry. Following this, completed a postdoctoral fellowship in Dr. Robert E.W. Hancock's laboratory, Centre for Microbial Diseases and Immunity Research, University of British Columbia. Here, my work focused on the design and development of novel anti-infective therapeutics and vaccine adjuvants, which are substances that are added to vaccine formulations to help amplify the immune response to the vaccine, for emerging pathogens. During this postdoctoral fellowship, my research focused on the investigation of emerging and re-emerging pathogens of importance to global public health, notably multi-drug resistance. These investigations fostered my commitment to both basic scientific research approaches and application of this research to public health in both high income and low- and middle-income regions. In 2009, I joined the National Institutes of Health (NIH) in Bethesda, MD, USA, as a Visiting Fellow to expand my expertise in the molecular mechanisms that underlie severe infections focusing on emerging and re-emerging viruses. Following my visiting fellowship, I served in multiple senior scientific capacities, including Principal Research Scientist (NIH Integrated Research Facility-Frederick) and Staff Scientist (Critical Care Medicine Department, NIH).

Research History

My research has contributed to our understanding of the complex mechanisms underlying emerging viruses, their transmission and the infections they cause. Research in my laboratory focuses on the circulation, transmission and clinical aspects of emerging viruses that pose the greatest threat to global human and animal health. My prior COVID-19 research includes:

- characterization of how SARS-CoV-2 manipulates human immune responses to cause severe disease in high-risk patient populations
- investigation of repurposed drugs as SARS-CoV-2 therapeutics through kinome analysis
- characterization of neurological and reproductive health complications in animal models of SARS-CoV-2 infections. Further, the animal models we are developing will allow us to inform how neurological manifestations associated with COVID-19 occur in humans.

Most Significant Contributions:

1. Orthopoxvirus research. I have extensive experience working with human orthopoxviruses, including monkeypox virus. This has included the first identification of Clade I monkeypox virus infections mediated by sexual contact in the Democratic Republic of the Congo.

a. Savinkina A, **Kindrachuk J**, Bogoch II, Rimoin AW, Hoff N, Shaw SY, Muyembe-Tamfum JJ, Mbala-Kingebeni P, Gonsalves GS. Lancet. [In Submission]

a. Kibungu EM, Vakaniaki EH, Kinganda-Lusamaki E...**Kindrachuk J***, Mbala-Kingebeni P*. Emerg Infect Dis. 2024. 30:172-176

b. Wahl V, Olson VA, Kondas AV, Jahrling PB, Damon IK, **Kindrachuk J***. J Infect Dis. 2023. Jiad516,

c. **Kindrachuk J***, Arsenault R, Kusalik A, Kindrachuk KN, Trost B, Napper S, Jahrling PB, Blaney JE. Mol Cell Proteomics. 2012. 11: M111.015701.

d. Okwor T, Mbala PK, Evans DH, **Kindrachuk J***. Clin Microbiol Infect. 2023: S1198-743X(23)00337-3.

2. Ebola virus research & support efforts. I have extensive experience in Ebola virus pathogenesis research and response efforts. These include the first temporal analysis of host transcriptome responses throughout the course of non-fatal EVD and investigation of post-recovery EBOV testicular persistence.

a. Schindell BG, Kangbai J, Fredborg B, Kowalec K, Shaw S, **Kindrachuk J***. BMJ Global Health. [In Revision]

b. Webb AL, Schindell B, Soule G, Siddik AB, Abrenica B, Memon H, Su R, Safronetz D, **Kindrachuk J***. NPJ Viruses. [Accepted]

a. Schindell BG, Kangbai JB, Shaw SY, **Kindrachuk J***. J Infect Public Health. 2024. 17:35-43

3. Coronavirus research. I have been extensively involved in coronavirus research since 2013 beginning with characterizing host responses to MERS-CoV infection and identification of novel

therapeutics and therapeutic targets. I have been actively involved in Covid-19 research and analysis and have served on multiple national panels as an virology and transmission expert.

a. Stein D, **Kindrachuk J**, McKinnon L, Reimer J, Bullard J, Van Caesseele P, Shaw S. Front Epidemiol. [Accepted]

b. Francis ME, Richardson B, McNeil M, Rioux M, Foley MK, Ge A, Pechous RD, **Kindrachuk J**, Cameron CM, Richardson C, Lew J, Cameron MJ, Gerdts V, Falzarano D, Kelvin AA. Sci Rep. [Accepted]

c. Escandón K, Rasmussen AL, Bogoch II, Murray EJ, Escandón K, **Kindrachuk J***. BMC Infect Dis. 21: 710

d. Francis ME, Goncin U, Kroeker A, Swan C, Ralph R, Lu Y, Falzarano D, Gerdts V, Machtaler S, **Kindrachuk J**, Kelvin AA. PLoS Path. 17: e1009705

4. Species-specific kinome analysis. I participated in the development of open-access kinome software, the Platform for Intelligent, Integrated Kinome Analysis.

a. Falcinelli, S., Gowen, B., Trost, B., Napper, S., Kusalik, A., Safronetz, D., Prescott, J., Johnson, R.F., Wahl-Jensen, V. Jahrling, P.B. and **Kindrachuk, J***. (2015) Characterization of the functional host response to Pichinde virus infection in the Syrian golden hamster. Mol Cell Proteomics. 14: 646-57.

b. Trost, B., **Kindrachuk, J.**, Scruten, E., Griebel, P., Kusalik, A. and Napper, S. (2013) Personalized profiles of cellular kinase activity: the kinotype. BMC Genomics 14: 854.

5. Influenza virus pathogenesis. I have investigated influenza virus pathogenesis extensively with a focus on influenza-bacterial co-infections.

a. Nickol, M.E., Lyle, S.M., Dennehy, B. and **Kindrachuk, J***. Dysregulated Host Responses Underlie 2009 Pandemic Influenza-Methicillin Resistant Staphylococcus aureus Coinfection Pathogenesis at the Alveolar-Capillary Barrier. Cells. 9: 2472

b. Nickol, M.E., Ciric, J., Falcinelli, S.D., Chertow, D.S. and **Kindrachuk, J*.** (2019) Characterization of Host and Bacterial Contributions to Lung Barrier Dysfunction Following 2009 Pandemic Influenza-Methicillin Resistant Staphylococcus aureus Co-infection. Viruses. 11: 116

Published Reports on COVID-19 Transmission:

1) CIHR-PHAC-CADTH – Best Brains Exchange – Transmission Routes for COVID-19: Implications for Public Health. Canadian Institutes of Health Research (CIHR); 2020 October. https://cihr-irsc.gc.ca/e/52238.html
2) Heating, Ventilation and Air Conditioning Systems in Public Spaces. Ottawa: Canadian Agency for Drugs and Technologies in Health (CADTH); 2020 June. (CADTH technology review). Heating, Ventilation, and Air Conditioning Systems in Public Spaces (cadth.ca)

National and International COVID-19 Committees:

1) Panel Member – CIHR Institute of Infection and Immunity Consultation on Variant Strains of SARS-CoV-2
2) Panel Member – CIHR-PHAC-CADTH – Best Brains Exchange – Transmission Routes for COVID-19: Implications for Public Health. Canadian Institutes of Health Research (CIHR); 2020 October. https://cihr-irsc.gc.ca/e/52238.html
3) Panel Member – Heating, Ventilation and Air Conditioning Systems in Public Spaces. Ottawa: Canadian Agency for Drugs and Technologies in Health (CADTH); 2020 June. (CADTH technology review). Heating, Ventilation, and Air Conditioning Systems in Public Spaces (cadth.ca)
4) Member – World Health Organization COVID-19 Solidarity Serology Study Group
5) Member – World Health Organization Ad Hoc Committee on COVID-19 Animal Models

2. COVID-19 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), was first designated as a Public Health Emergency of International Concern (PHEIC) by the World Health Organization on 30 January 2020 following the recommendations of the Emergency Committee [1]. SARS-CoV-2 was first identified by genome sequencing in January 2020 following a atypical pneumonia of unknown origin was identified in a cluster of cases with epidemiological links to the Huanan seafood market, a live animal market, in Wuhan, Hubei Province, China [2]. Early analysis identified similarity of SARS-CoV-2 to bat coronaviruses, suggesting that SARS-CoV-2 may have originated as the result of a natural spillover event from bats to humans in late 2019 [3-5]. We reviewed SARS-CoV-2 emergence and the spillover potential of this and related betacoronaviruses into multiple animal species [6]. SARS-CoV-2 is the seventh coronavirus that has emerged in humans, with SARS-CoV, SARS-CoV-2 and MERS-CoV all sharing bats as their likely reservoir host in the wild [7, 8].

COVID-19 illness frequently resembles an influenza-like illness with primary symptoms including fever, cough, malaise, myalgia, headache, and taste and smell disturbance [9, 10]. Disease severity ranges from asymptomatic to severe and critical disease with the highest risks of severe disease associated with age and underlying health conditions with age being the strongest risk factor for severe disease. The risk increases for people >50 years of age with the highest risk found in those ≥ 85 years (**Figure 1**) [11]. There are also underlying medical conditions that are associated with increased risk for severe COVID-19. The US Centers for Disease Control and

Prevention have outlined associations between underlying health conditions and COVID-19 severity through systematic review of available evidence. Strong evidence for higher risk of severe COVID-19 outcomes were associated with cancer, cerebrovascular disease, chronic kidney disease, chronic lung disease, chronic liver disease, cystic fibrosis, diabetes mellitus (type 1 and type 2), learning disabilities, intellectual and developmental disabilities, heart conditions, HIV, mental health disorders, neurological conditions limited to dementia, obesity, primary immunodeficincies, pregnancy, physical inactivity, smoking (current and former), tuberculosis and use of immunosuppressive medications.

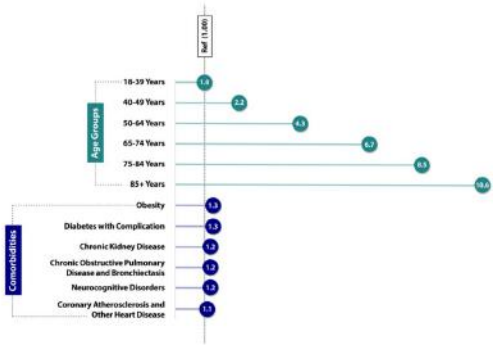
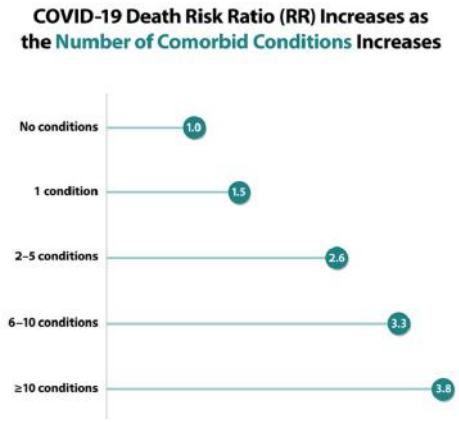


Figure 1: COVID-19 death risk ratios. Based on age groups and underlying health conditions [12].

It must also be appreciated that multiple underlying medical conditions further increases the risk of severe COVID-19 (**Figure 2**) [13, 14]. Those living with disabilities have an icreased likelihood of severe disease as compared to those living without disabilities [15-17]. This includes higher rates of living in congregate settings, which can increase the overall risk of infection, increased rates of chronic health conditions, which increases the risk for severe disease, and greater barriers for health care access barriers, which can increase the risk for worse outcomes. There is also a disproportionate burden of COVID-19 within racialized and marginalized communities. Race and ethnicity are also linked to increased risk of SARS-CoV-2 infections as well as risk for severe or fatal COVID-19. The US CDC has provided continued updates on this and provides the following risk factors as compared to those for white, non-Hispanic persons [18]. Moreover, data has also shown that people from racial and ethnic minority groups are more likely to die from COVID-19 at younger ages as compared to non-Hispanic, white people [19]. The complexity of susceptibility to severe COVID-19 is further demonstrated by the increased frequency of underlying chronic medical conditions within racial or ethnic minority groups. Increased risks have been associated with certain social determinants of health including discrimination, healthcare use and access, occupations, housing, and educational, income, and wealth gaps [20].

A.



B.

Rate ratios compared to White, Non-Hispanic persons	American Indian or Alaska Native, Non-Hispanic persons	Asian, Non-Hispanic persons	Black or African American, Non-Hispanic persons	Hispanic or Latino persons
Cases[1]	1.5x	0.7x	1.1x	1.5x
Hospitalization[2]	3.1x	0.8x	2.5x	2.3x
Death[3]	2.7x	0.8x	1.7x	1.1x

Race and ethnicity are risk markers for other underlying conditions that affect health, including socioeconomic status, access to health care, and exposure to the virus related to occupation, e.g., frontline, essential, and critical infrastructure workers.

Figure 2: COVID-19 death risk ratios. Based on (A) number of underlying health conditions and (B) COVID-19 rate ratios across racialized communities [12, 18].

Groff and colleagues performed a systematic review of short- and long-term postacute sequelae (PASC) for COVID-19 [21]. The authors review suggested the PASC were dependent on time from infection, organ systems and tissue affected, vaccination status, variant of the virus, and geographic region. A total of 57 studies (250,351 survivors) met the authors' inclusion criteria with the following demographics: mean age of 54.4 years, 56% male, 79% hospitalized and 79% from high-income regions. At least one PASC was reported in 54%, 55% and 54% of patients at 1 month, 2-5 months and ≥6 months. The most prevalent sequelae were chest imaging abnormality (62.2%), generalized functional impairments (44%), fatigue or muscle weakness (37.5%), generalized anxiety disorder (29.6%), and difficulty concentrating (23.8%). Logue and colleagues assessed longer term PASC through a longitudinal prospective cohort study that utilized data from 177 patients between three to nine months following disease onset between August to November 2020 [22]. This was a descriptive analysis only with no statistical testing due to limited group numbers. Mean age was 48 years, 57.1% of participants were women and asymptomatic illness, mild illness and illness requiring hospitalization comprised 6.2%, 84.7% and (9%) of the patients. Fatigue and loss of smell were each reported by 13.6% of patients and 7.9% of patients reported negative impacts on at least one daily living activity.

Regarding vaccination and COVID-19 up to mid-to-late 2021, we can consider the comparison of daily COVID-19 cases during the third pandemic wave (Delta variant) as compared to

vaccination rates using data from Ontario during this time frame (**Figures 3**). While the trajectory of cases during the third wave were already increasing rapidly by 01 April 2021, vaccinations reached 2 million doses by this same date and doubled to 4 million by 22 April 2021. Thus, vaccinations were increasing significantly as Delta was beginning to move through the population and made up <20% of cases by the end of April. Taken together, the impact of vaccination on Delta transmission cannot be discounted given that Delta transmission began to decrease as vaccination continued to increase across the provincial population. Further, the impacts of Omicron on cases and severe outcomes in long term care facilities, a population that is highly vulnerable to severe COVID-19 and was disproportionately impacted during early pandemic waves, remained low following vaccination campaigns. This is complemented by assessments of vaccine effectiveness by Buchan and colleagues where VE against symptomatic Delta infection was 89% (95%CI, 86-92%) from 7-59 days following a second dose and dropped to 80% (95%CI, 74-84%) after ≥240 days [23].

Madewell and colleagues recently assessed household secondary attack rates by SARS-CoV-2 variant and vaccination status in a systematic review and meta-analysis comparing 126 total studies representing 1,437,696 contacts from 35 countries [24]. Overall, their analysis demonstrated that while household contacts exposed to Delta or Alpha variants were at increased risk of infection compared to the ancestral virus, vaccination reduced susceptibility to infection and infectiousness. In their assessment, the authors provided vaccine effectiveness estimates for susceptibility (VEs,p), infectiousness (VEi,p) and total vaccine effectiveness (VEt,p). Secondary attack rates (SARs) can be used to estimate the protective effectiveness of a vaccine in vaccinated susceptible contacts compared to unvaccinated susceptible contacts who are exposed to an infected index case (VES,p). Vaccine effectiveness in reducing infectiousness (VEI,p) can be assessed by comparing SARs from vaccinated and from unvaccinated index cases to household contacts. Lastly, these two values can be used to calculate the total vaccine effectiveness (VET,p), or the combined effect of direct vaccine protection and indirect vaccine effectiveness. Lyngse and colleagues also recently examined the impacts of vaccination on Delta and Omicron transmission [25]. Here, fully vaccinated individuals as well as those that received boosters were generally less susceptible to Delta and Omicron infection compared to unvaccinated individuals and that unvaccinated individuals were more likely than vaccinated individuals Delta and Omicron infections to transmit infection to their household contacts.

Overall, the authors found that household SARs from fully vaccinated index cases were lower than from unvaccinated index cases. Fully and partially vaccinated household contacts were less susceptible to SARS-CoV-2 infection than unvaccinated contacts. SARs for Delta and Alpha were significantly higher than estimates for the original wild-type variant. The authors also found lower transmission to household contacts from fully vaccinated index cases than from unvaccinated index cases, but not from partially vaccinated index cases. These results were in line with an observational cohort study where two doses of BNT162b2 (Pfizer) or ChAdOx1 (AstraZeneca) reduced onward transmission of Delta, but by less than Alpha, and the impact of vaccination against onward transmission waned over time [26]. Madewell and colleagues did

address factors including differences in the study population (e.g., age, comorbidities, serostatus), viral characteristics, vaccine type, time period defining vaccination status, intensity of the epidemic, community behavior, and use of nonpharmaceutical interventions (masks, social distancing) precluded their ability to make comparisons of vaccine effectiveness across studies.

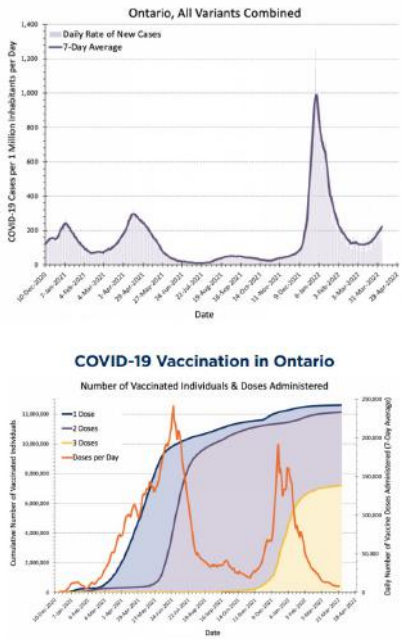


Figure 3: Comparison of daily COVID-19 cases as compared to vaccination rates in Ontario (Courtesy of Ontario Science Table).

In an observational study published 27 April 2022 from Suthar and colleagues, the authors evaluated the impact of COVID-19 vaccination on mortality and incidence in the US [27]. Using data representing 300 million people (80% of the US population), the authors found that increased vaccination coverage was associated with a reduced incidence of COVID-19 cases and mortality. In the first year of vaccine roll-out, the authors found that a 10% improvement in vaccination coverage was associated with an 8% reduction in mortality rates and a 7% reduction in case incidence and continued population level protection against death and reductions in population level protection against infection during the predominance of the Delta variant.

Comparative analyses of Omicron severity to prior SARS-CoV-2 variants have primarily focused on Delta. There is broad consensus that the Omicron variant is associated with a lower risk of severe disease. In their B.1.1.529 (Omicron) risk assessment, Public Health Ontario cautioned that: “There remains insufficient data to comment on hospitalization outcomes, including progression of severity of illness, complications, and mortality” [28]. The US Centers for Disease Control and Prevention also assessed trends in COVID-19 severity across different periods of high transmission, including Omicron [29]. Omicron resulted in the highest number of COVID-19 associated emergency department visits and hospital admissions since the beginning of the pandemic; however, disease severity appeared lower compared to prior periods of high disease-transmission. Importantly, the authors caution that this likely relates to multiple factors

including increases in vaccination coverage among eligible persons, and the use of vaccine boosters among recommended subgroups. While overall vaccinations continued to increase from the Delta to Omicron waves, booster doses were significantly higher during the Omicron period compared to Delta, with 78 million persons and 1.6 million persons, respectively. Additionally, infection-mediated immunity and the potential lower virulence of the Omicron variant have likely also contributed.

SARS-CoV-2 has resulted in unprecedented transmission dynamics across populations. If we look at cases of influenza-like illness during the 2018-2019 influenza season in Canada, we can see a single peak of illness for both the 2018-2019 season as well as in the five-year average (from 2018 to 2019; **Figure 4**) [30]:

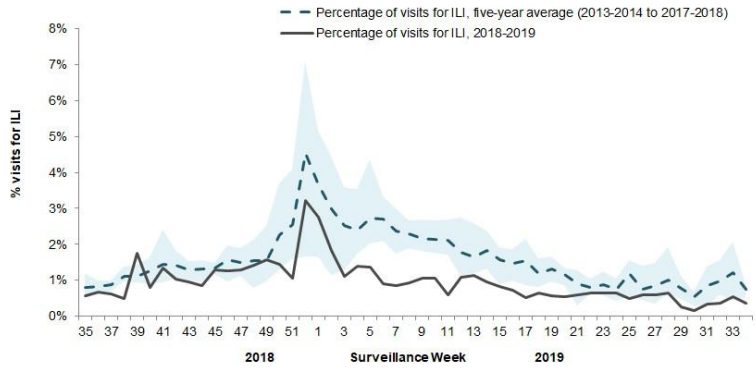


Figure 4: Percentage of visits for ILI reported by sentinels by report week, Canada, weeks 2018-35 to 2019-34 [30].

From 2020-2022, there have been multiple waves of distinct SARS-CoV-2 variants that have moved across the globe, including Canada. During the 2018-2019 influenza season in Canada, there were a total of 3,657 influenza-associated hospitalizations, 613 ICU admissions and 224 deaths reported. From 02 January 2022 to 14 April 2022, there were 7,522 deaths due to COVID-19 [31].

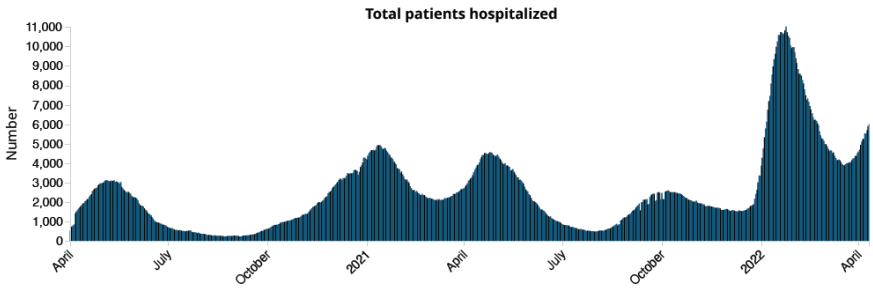


Figure 5: Daily number of hospital beds occupied by COVID-19 patients. As of April 11, 2022 [31].

Longitudinal COVID-19 hospitalization data is presented in **Figure 5**. This data demonstrates the magnitude of difference in COVID-19 hospitalizations versus those seen during a typical

influenza season, such as the 3,657 **total hospitalizations** due to influenza-like illness during the 2018-2019 influenza season. Further, if we compare the national ICU patient data for COVID-19 to that from the 2018-2019 influenza season, we can clearly see that while total ICU admissions for influenza were 613 for the 2018-2019 season, there were multiple periods during the COVID-19 pandemic where ICU patients neared or exceeded 1,000 total nationally (**Figure 6**):

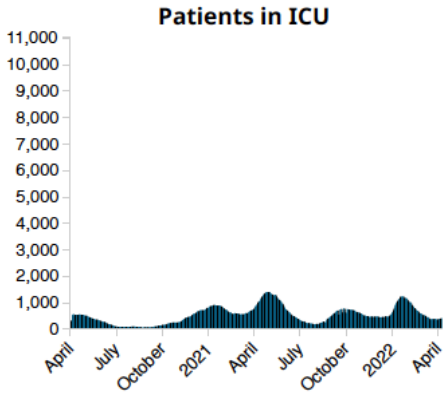


Figure 6: Daily number of ICU beds occupied by COVID-19 patients. As of April 11, 2022 [31].

In addition, we can also consider that multiple waves of COVID-19 have been due to distinct SARS-CoV-2 variants. Successive waves with new virus variants have resulted in similar or increased healthcare system burden as evidenced by total hospitalizations and/or ICU admissions throughout the pandemic. The Omicron BA.1 variant, which is associated with lower risks of severe disease than seen with the Delta variant, still resulted in more overall hospitalizations than the Alpha and Delta waves combined (waves 2 and 3) and similar ICU admissions to that seen during the Delta wave (wave 3), and more than see during the Alpha wave (wave 2).

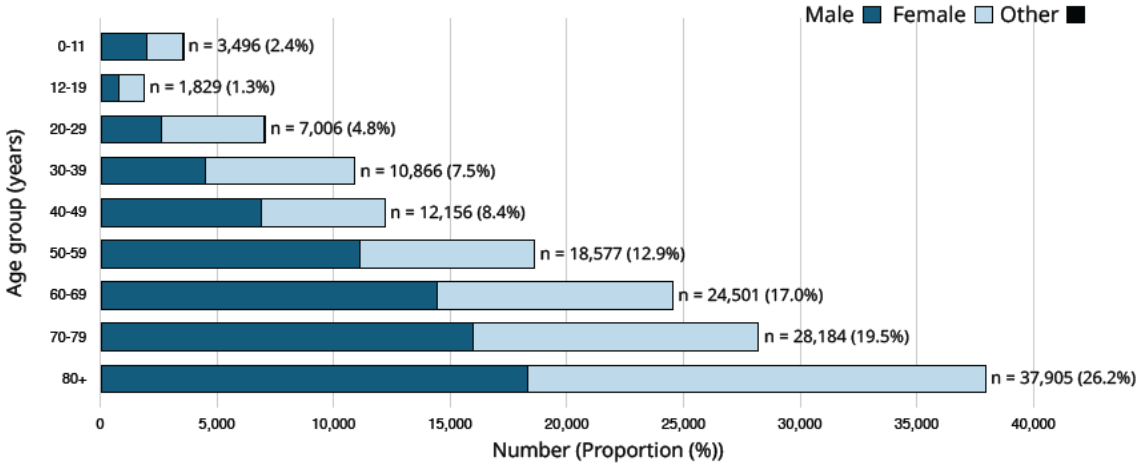


Figure 7: Age and gender distribution of COVID-19 cases hospitalized in Canada. As of April 8, 2022 [31].

The total hospitalizations for those aged 0-11 (n=3,496) were nearly identical to the total influenza-like illness hospitalizations **across all age groups** for the 2018-2019 season (n=3,657; including a total of 1,352 pediatric hospitalized cases; **Figure 7**). COVID-19-related ICU admissions for this age group were 330 (613 total across all age groups for the 2018-2019 influenza season) with 25 deaths. During the 2018-2019 influenza season, there were a total of 271 ICU admissions and 10 deaths reported for pediatric patients. Overall, these data demonstrate the unprecedented public health impact of the COVID-19 pandemic.

Stoddard and colleagues have modelled COVID-19 burdens in the US following endemicity [32]. The authors' analysis suggests that the annual burden of endemic COVID-19 within the United States would be in the hundreds of thousands, multiple orders of magnitude above annual influenza fatalities within the US [33]. It is of note that annual **global** influenza fatalities range in the hundreds of thousands (291,243–645,832) [34].

The Ontario Science Table (https://covid19-sciencetable.ca/ontario-dashboard/) provided continual updates on COVID-19 risks by vaccination status. Protection against hospitalization and ICU admission remained >70% past the end of 2021 with at least two doses of vaccine (Figure 8):

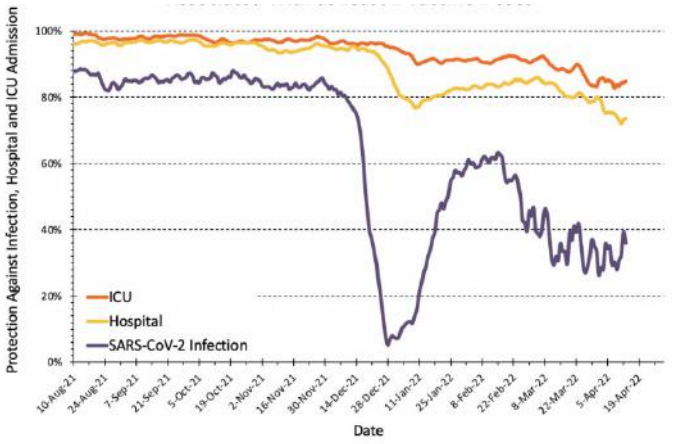


Figure 8: Protection against infection, hospitalization and ICU admission associated with at least two vaccine doses (Courtesy of Ontario Science Table).

Further, data was broken down comparing these risks against vaccination status (**Figure 9**):

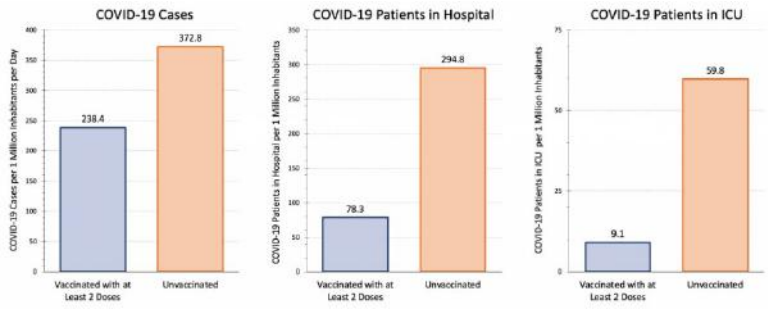


Figure 9: Protection against infection, hospitalization and ICU admission by vaccination status (Courtesy of Ontario Science Table).

While it is correct that vaccine effectiveness against SARS-CoV-2 infection does wane over time and has been further impacted by the immune evasion activities of the Omicron variant of concern, vaccination continued to provide robust protection against hospitalization and severe disease. This is also demonstrated in the Ontario Science Table longitudinal assessments of COVID-19 risks by vaccination status (**Figure 10**).

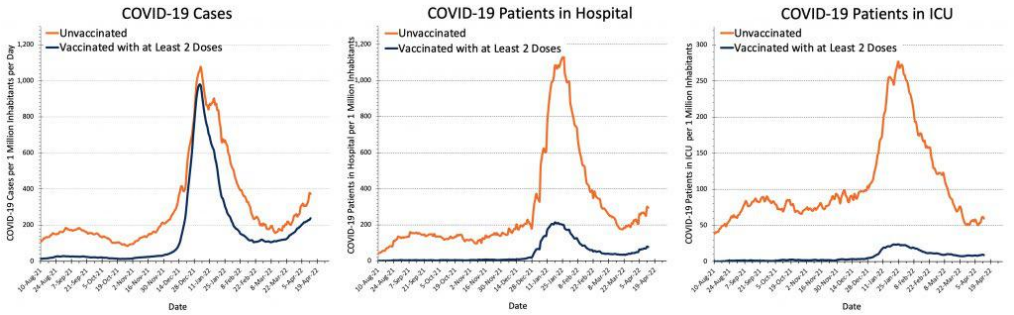


Figure 10: Longitudinal assessment of protection against infection, hospitalization and ICU admission by vaccination status (Courtesy of Ontario Science Table).

3. SARS-CoV-2 Infection and Vaccination

Regarding vaccine effectiveness against symptomatic infection and onward transmission, vaccination substantially reduced the risk of infection with the Delta variant, and individuals who are not infected cannot transmit the disease. Thus, transmission is by definition affected by vaccination. Further, there is evidence that when infection does occur in vaccinated persons, there is reduced transmission to others.

Nasreen and colleagues assessed various protective benefits of vaccination against SARS-CoV-2 variants [35]. Their analysis included community-dwelling Ontarians aged ≥16 years who had symptoms consistent with, or a severe outcome attributable to, COVID-19, and who were tested for SARS-CoV-2 between 14 December 2020 and 3 August 2021. Cases of Delta infections were younger, more likely to reside in the Central West region, more likely to occur later in the study period, less likely to have any comorbidities, and more likely to reside in neighbourhoods with lower household income and greater proportions of essential workers than cases of other variants of concern and non-variant of concern SARS-CoV-2 infections.

The mRNA vaccines provided strong protection from infection after administration of the second dose. Here, vaccine effectiveness increased to 95% (95% CI, 91–97%) for Moderna Spikevax (mRNA-1273) and 92% (95% CI, 90–94%) for Pfizer/BioNTech. Both mRNA vaccines were superior to the AstraZeneca adenovirus vaccine after two doses (**Figure 11**).

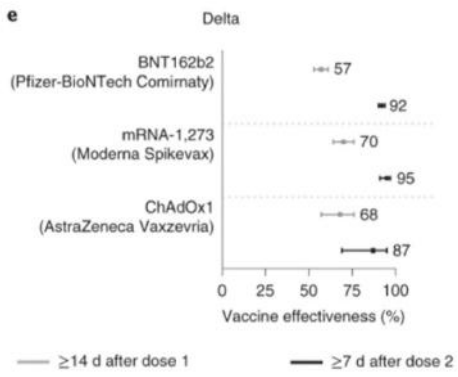


Figure 11: Vaccine effectiveness against infection with the SARS-CoV-2 Delta variant [35].

For vaccine effectiveness against hospitalization and death, second dose administration of the mRNA vaccines resulted in >95% protection (**Figure 12**). Both mRNA vaccines were superior to the AstraZeneca adenovirus vaccine after two doses.

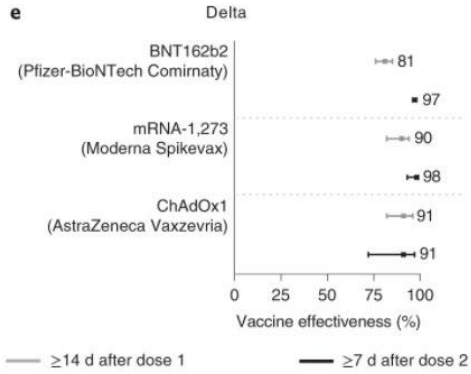


Figure 12: Vaccine effectiveness against hospitalization and severe outcomes due to the SARS- CoV-2 Delta variant [35].

In age-group-stratified analyses, vaccine effectiveness against symptomatic infection caused by all variants of concern were predominantly lower or similar in older adults (age ≥60 years) compared with younger individuals (age <60 years) after partial vaccination but increased to levels comparable to those in younger individuals after the second dose. Vaccine effectiveness was higher against hospitalization and death than against symptomatic infection for both older and younger adults.

Buchan and colleagues assessed the effectiveness of vaccination against symptomatic Delta infections and severe outcomes [23]. Using Ontario data, the authors compared the effectiveness of homologous or heterologous Canadian vaccination schedules for protection from symptomatic infection and severe outcomes resulting from the Delta and Omicron variants. Vaccine effectiveness against severe outcomes (hospitalization or death) were similar for Delta (99%) and Omicron (95%) seven days after a third vaccine dose. Vaccine effectiveness against symptomatic

infection by the Delta variant declined steadily over time but recovered to 97% at least seven days following a third vaccine dose.

It must also be appreciated that infection does not correlate directly with concomitant transmission of virus. For example, recent analysis of viral load data in Delta breakthrough infections by Puhach et al demonstrated that vaccinated individuals had significantly lower RNA genome copies and infectious virus titers compared to unvaccinated subjects while also clearing virus faster [36]. This data suggests that even in the event of a breakthrough infection, onward transmission of virus from a vaccinated, infected individual is likely reduced as compared to an unvaccinated, infected individual.

Vaccine effectiveness data from Canada demonstrated that vaccination provides strong protection against both infection as well as hospitalization and fatal disease and waning protection from infection is strongly restored after a third dose. Thus, vaccination provides a strong layer of protection, even in the absence of NPIs; however, this must be weighed against the time since the second dose, administration of a third dose, and underlying conditions related to greater risk of severe disease. Vaccination was highly beneficial for reducing the burden of disease attributable to Delta.

4. **Vaccine Safety Considerations:**

Amalgated vaccine adverse event data in Canada is publicly available through the Government of Canada: COVID-19 vaccine safety: Report on side effects following immunization - Canada.ca. All data in the following sections pertaining directly to national vaccination and adverse event data is from this website specifically.

As of 03 December 2024, 105,016,456 vaccine doses have been administered in Canada. Longitudinal vaccine adverse events reported are reported in **Figure 13**. Number of vaccine adverse events by vaccine type are presented in **Figure 14** and all vaccine doses administered by vaccine type is presented in **Figure 15.**. Among these, there have been 58,712 adverse events reported (up to 05 January 2024) which equates to 0.06% or ~6 adverse events/10,000 people vaccinated. Of the total adverse events reported, 11,702 of the total 58,712 adverse events were considered serious (0.011%; 11/100,000 doses). Adverse events are defined as an unfavourable or unintended sign (e.g. skin rash), abnormal laboratory finding, symptom or disease. Serious adverse events are subdivided from adverse events and are defined as those that result in the following: death, is life-threatening event (patient at real risk of death at the time of the event/reaction), in-patient hospitalization or prolongation of existing hospitalization, persistent or significant disability/incapacity, or congenital anomaly/birth defect. Adverse events are reported by individuals through an Adverse Events following Immunization (AEFI) Form completed by a doctor, nurse, or pharmacist.

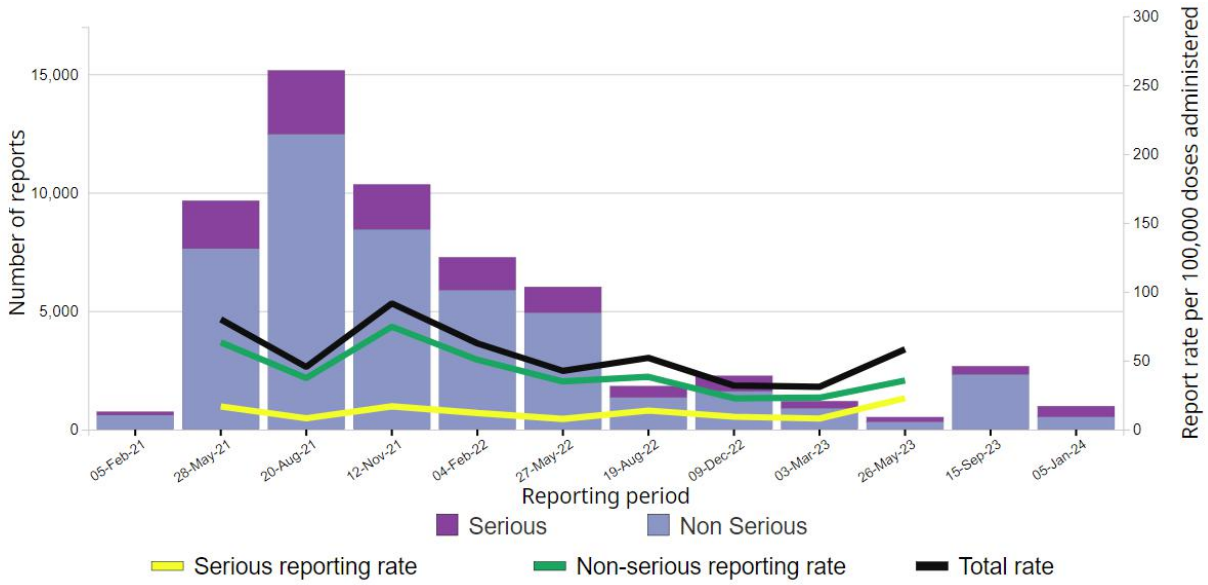


Figure 13: Quarterly number and rate of COVID-19 vaccine adverse event reports received for all people and total doses administered per reporting period (up to 05 January 2024; total events = 58,712).

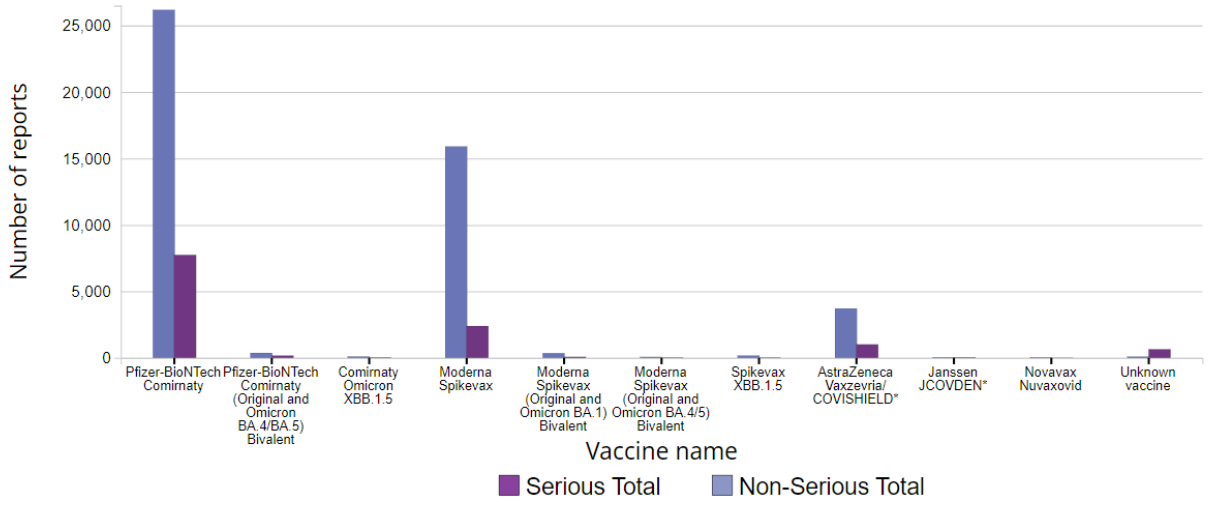


Figure 14: Number of adverse event reports received for all people by vaccine name and all doses (up to 05 January 2024.

Vaccine group	Vaccine product	Total doses
Vaccines with the XBB.1.5 strain	Pfizer-BioNTech Comirnaty XBB.1.5 12 years and older	2,178,196
	Pfizer-BioNTech Comirnaty XBB.1.5 5-11 years	14,288
	Pfizer-BioNTech Comirnaty XBB.1.5 6 months-4 years	1,708
	Pfizer-BioNTech Comirnaty XBB.1.5 (not specified)	1,186,048
	Moderna Spikevax XBB.1.5	3,198,765
	Novavax Nuvaxovid XBB.1.5	448
	Subtotal	6,579,453
Pfizer-BioNTech Comirnaty vaccines with the original strain	Pfizer-BioNTech Comirnaty (ages 12 years and older)	57,423,206
	Pfizer-BioNTech Comirnaty (ages 5 to 11 years)	3,364,743
	Pfizer-BioNTech Comirnaty (ages 6 months to 4 years)	39,053
	Pfizer-BioNTech Comirnaty Bivalent (BA.4/BA.5)	5,615,727
	Subtotal	66,442,729
Moderna Spikevax vaccines with the original strain	Moderna Spikevax	25,015,836
	Moderna Spikevax (ages 6 months to 5 years)	309,977
	Moderna Spikevax Bivalent (BA.1)	4,040,394
	Moderna Spikevax Bivalent (BA.4/BA.5)	274,634
	Subtotal	29,640,841
Other vaccines	AstraZeneca Vaxzevria/COVISHIELD	2,811,963
	Janssen Jcovden*	23,875
	Novavax Nuvaxovid	37,343
	Medicago Covifenz*	863
	Subtotal	2,874,044
Unknown	Vaccine unknown	68,565
All vaccines	All vaccines	105,605,632

Figure 15: All vaccine doses administered in Canada by product (as of 25 February 2024).

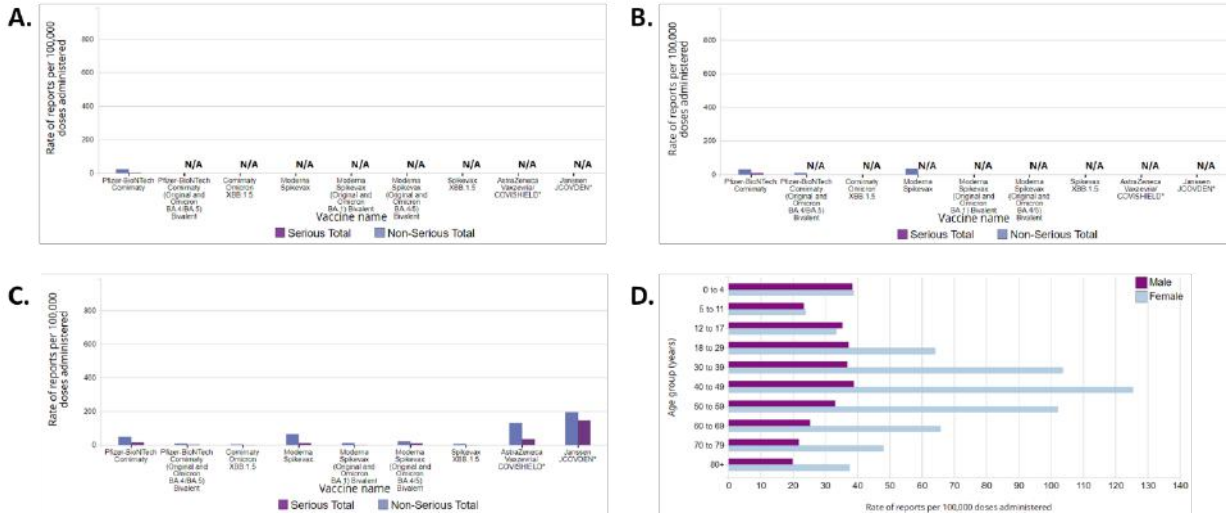


Figure 16: Vaccine adverse events by product and dose. Total rate of events presented by age group: A) 5-11 years. B) 12-17 years. C) ≥18 years. D) Rates of adverse events stratified by age and sex (as of 25 February 2024).

From the Canadian data presented in **Figure 16**, the rate of reported adverse events was highest in those 40-49 years (0.085%; 84.6/100,000 doses) and 30-39 years (0.072%; 72.3/100,000 doses). The rate of adverse event reports was lowest among those 5-11 years. Reported events were higher for females than males (75.9/100,000 doses vs 31.5/100,000 doses); however, reporting rates were similar between females and males in those <18 years. Adverse event types reported by rate are presented in **Figure 17**.

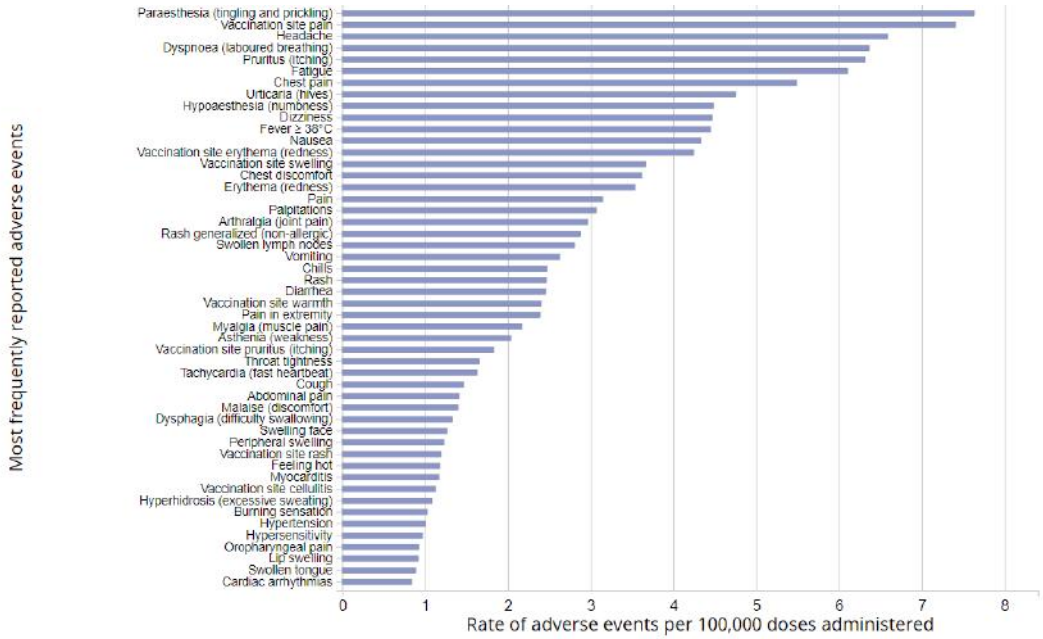


Figure 17: Rates of the most frequently reported vaccine adverse events reported across all vaccine types (data as of 05 February 2024).

Rate data for adverse events of special interest (AESI) are provided in **Table 1**. These include pre-specified medically-significant events that have the potential to be casually associated with vaccination. However, it should be noted that AESI can include serious and non-serious events that may be associated with SARS-CoV-2 infection or with vaccines in general.

AESI Category	AESI	Total reporting rate per 100,000 doses administered
Auto-immune diseases	Guillain-Barré Syndrome[1]	0.02
	Thrombocytopenia (low blood platelets)[1]	0.19
	Subtotal	0.22
Cardiovascular system	Cardiac arrest	0.06
	Cardiac failure	0.10
	Myocardial infarction (heart attack)	0.15
	Myocarditis/Pericarditis[1] (inflammation of the heart muscle and lining around the heart)	1.17
	Subtotal	1.49
Circulatory system	Cerebral venous (sinus) thrombosis	0.03
	Cerebral thrombosis	0.02
	Cutaneous vasculitis	0.05
	Deep vein thrombosis	0.39
	Embolism	0.02
	Haemorrhage (bleeding)	0.31
	Pulmonary embolism	0.56
	Thrombosis (blood clot)	0.36
	Thrombosis with thrombocytopenia syndrome (blood clot with low platelets)[1]	0.08
	Subtotal	1.83
Hepato-gastrointestinal and renal system	Acute kidney injury	0.08
	Glomerulonephritis (kidney inflammation) and nephrotic syndrome (kidney disorder)	0.03
	Liver injury	0.04
	Subtotal	0.14
Nerves and central nervous system	Bell's Palsy[1]/facial paralysis	0.21
	Cerebrovascular accident (stroke – includes ischemic and hemorrhagic strokes)	0.29
	Transverse myelitis (inflammation of spinal cord)[1]	0.02
	Subtotal	0.52
Other system	Anaphylaxis[1]	0.74
	COVID-19[2]	1.30
	Multisystem inflammatory syndrome[1]	0.02
	Subtotal	2.06
Pregnancy outcomes[3]	Fetal growth restriction	N/A
	Spontaneous abortion	0.09
	Subtotal	0.10
Respiratory system	Acute respiratory distress syndrome	0.01
	Subtotal	0.01
Skin and mucous membrane, bone and joints system	Chilblains	0.03
	Erythema multiforme (immune skin reaction)	0.06
	Subtotal	0.10
All AESI categories	**Total**	**6.46**

Table 1: Rate of reported AESI by all vaccine types (data up to 05 January 2024; total AESI = 6,781).

For specific safety signals that have been confirmed for potential causal association with COVID-19 vaccination in Canada, two have been confirmed. For thrombosis with thrombocytopenia syndrome (TTS), including vaccine-induced prothrombotic immune thrombocytopenia, 89 TTS reports have been identified as of 05 January 2024 with 56/89 reported following AstraZeneca Vaxzevria/COVISHIELD vaccination, 24/89 following Pfizer-BioNTech Comirnaty, and 8 following Moderna Spikevax administration. According to the Advisory Committee on Causality Assessment, 59/84 total TTS reports as of 31 October 2022

were found in those ≥50 years (25/85 in those <50 years). Viral vector-based vaccines accounted for 53/84 total TTS events reported with mRNA vaccines accounting for 31/84 events. Of these, 5/31 events related to mRNA vaccines were found among those <50 years. It is important to note that of the 84 TTS events reported here, 37/84 were consistent with causal association to vaccination and all others being indeterminate or inconsistent.

The second signal was myocarditis/pericarditis with 1231 total reports including 725/1231 following Pfizer-BioNTech Comirnaty and 465/1231 following Moderna Spikevax. For the Pfizer-related symptoms, the median age was 26 years (range 6-88 years) and symptom onset between 1 minute and 155 days post-vaccination. More than half of those were in males (474/725 events) and a median age of 21 years. Females comprised 243/725 events with a median age of 39 years. Most events followed second dose administrations (392/725 events) as compared to first dose (286/726). Median age for Moderna Spikevax was similar at 29 years and overrepresented by males as compared to females. Symptom onset was similar.

Finally, for deaths reported as adverse events, a total of 488 reports have been made in Canada up to 05 January 2024. Of these, 131 cases had missing information and could not be included in causality analysis. Of the remaining 357 cases, 4 deaths among the TTS reports were consistent with a causal association with vaccination while the remaining 353 cases were either indeterminate for causality, inconsistent with causality, or unclassifiable due to a lack of data. This results in 4 deaths total across all adverse events reported (4/58,712 events; 0.007%) and all vaccination doses administered (4/105,016,456 total doses; 0.000004%) in Canada. Mercade-Besora and colleagues recently investigated the impacts of COVID-19 vaccination on post-COVID thromboembolic and cardiovascular complications using electronic health data from the UK, Spain, and Estonia [37]. Through this, health data from >10 million vaccination and >10 million unvaccinated individuals were analyzed. Interestingly, the authors found that vaccination was associated with reduced risks of acute and post-acute COVID-19-related venous thromboembolism, arterial thromboembolism, and heart failure. The authors postulated that this was due to the reduced disease severeity associated with breakthrough infections in vaccinated individuals as compared to those infected and unvaccinated.

It is important to note that data from Canada is similar to that across other global locations. A recent systematic review and from Xu and colleagues using meta-analysis of observational epidemiological data found that there were a broad spectrum of adverse events associated with COVID-19 vaccines with most being transient, self-limiting, and mild to moderate. Similar to Canada COVID-19 vaccination data, adverse events were highest among younger adults and women. Importantly, they also found that those with prior SARS-CoV-2 infection were more likely to experience adverse events following vaccination [38]. Rosenblum and colleagues analysed data from the US Vaccine Adverse Event Reporting System (VAERS) and v-safe (passive and active reporting systems, respectively) to assess COVID-19 vaccine safety data over the first six months of the US vaccination program (14 December 2020-14 June 2021) [39]. Of note, this analysis focused on data from days 0-7 post-vaccination only to look for

reactogenicity, severity, and overall health impacts. A total of 298 792 852 doses of mRNA vaccines were administered during the study period with 340,522 reports processed in VAERS. Of these, 313,499/340,522 reports (92.1%) were non-serious, 22,527/340,552 (6.6%) were serious (non-death), and 4,496/340,522 (1.3%) were deaths. Of note, VAERS is a passive reporting system that while can function as an early signal warning system, it cannot be used to prove causality. In contrast to the Canadian adverse event reporting system, anyone can submit a report to VAERS including healthcare professionals, vaccine manufacturers, and the general public [40]. Through v-safe data, more than 50% of all participants (7,914,583) self-reported local and systemic reactogenicity, more frequently after dose two than after dose one. This included injection-site pain, fatigue, and headache after either dose one or two. Reactogenicity was reported most frequently the day after vaccination; most reactions were mild. Less than 1% of participants reported seeking medical care after vaccination after dose one or dose two. This data is in agreement that most reported adverse events following COVID-19 vaccination are mild and short in duration [41-44].

Sadarangani and colleagues investigated COVID-19 vaccine safety during pregnancy through the Canadian National Vaccine Safety network cohort study [45]. This observational cohort study was part of the Canadian Immunization Research Network and included seven provinces and territories. Participants were pregnant and non-pregnant females of 15-49 years and having received a COVID-19 vaccine dose in the prior seven days. The control population had the same demographics but were unvaccinated. By 04 November 2021, the study surveys included >190,000 women having received a first vaccine dose; >94,000 had received a second dose. Significant health events included the following: new or worsening health events following vaccination sufficient to cause work or school absenteeism, medical consultation, or prevent daily activities. Overall, 4% of vaccinated pregnant females reported a significant health event after dose one and 7.3% after dose two; pregnant unvaccinated females reported 3.2%. Two doses of Moderna Spikevax (mRNA-1273) resulted in increased odds of a significant health event within one week of the second vaccine dose compared to pregnant unvaccinated controls; however, this was not found with a single dose of Moderna Spikevax or any dose of Pfizer BioNTech Comirnaty. Pregnany vaccinated femailes had decreased odds of a significant health event compared to non-pregnant vaccinated females across either vaccine type and dose. Overall, the authors interpreted this data as providing strong evidence for a good safety profile for mRNA vaccines during pregnancy. The authors reference the largest study of COVID-19 vaccine reactogenicity during pregnancy in the US using the v-safe registry [46]. The study included ~30 000 pregnant individuals and who had received Pfizer BioNTech Comirnaty or Moderna Spikevax. Here, pregnancy correlated with higher rates of injection site pain (92% after dose two), fatigue (72%), headache (55%), myalgia (54%) and fever or chills (35–37%), and higher rates of adverse events following dose two. An additional study from 2021 comparing adverse events in vaccinated and unvaccinated pregnant females reported higher rates of adverse events in pregnant people after dose two of an mRNA COVID vaccine where 2.5% of pregnant females sought medical care after their second vaccine dose, compared to 1.5% in the Sadarangani study.

The authors noted that prior studies of influenza vaccines using a similar methodology also reported higher rates of adverse events following vaccination in vaccinated versus unvaccinated control participants [47].

Thomas and colleagues analyzed the safety and efficacy of the Pfizer Comirnaty vaccine during the first six months of two-dose vaccination for those ≥16 years [48]. Safety data for those 12-15 years could not be assessed in this study due to the timing of participant enrollment for this age cohort and focus on six months post-vaccination data. It should be noted that all patients were healthy (required for inclusion). Data was accrued by time post-vaccination (either first or second dose): days 0-7 (local reactions, systemic events, use of antipyretics or pain medication), adverse events (following first dose and including one month post-second dose), and severe adverse events (following first dose and through one and six months post-second dose). The authors found that reactogenicity within the first week of either first or second dose as well as adverse event reporting through six months were similar to prior analyses with no new additional safety signals.

Focused systematic review of COVID-19 vaccine safety and effectiveness data among children has also been undertaken. Notably, Piechotta et al. found that crude event rates for deaths in unvaccinated children were <1 case per 100,000 children, and no events were reported for vaccinated children [49]. The authors found no increased risk of serious adverse events reported in real-life observations. Myocarditis risk was determined as uncertain. Local reaction risks for one dose and two doses were both ~2 cases per 100,000 doses administered. The authors' interpretation of these findings was that children aged 5–11 years, mRNA vaccines are moderately effective against omicron variant infections but likely provide good protection against COVID-19 hospitalisations. While vaccines were reactogenic they were considered to probably be safe.

5. Conclusion

In reviewing the transcript of the On Point with Alex Pierson podcast, Dr. Bridle makes a number of statements on the efficacy of vaccines including:

- The "spike protein on its own is almost entirely responsible for the damage to cardiovascular system if it gets into circulation";
- "the spike protein gets into the blood, circulates through the blood in individuals over serveral days post vaccination. It accumulates, once it gets into the blood and accumulates in a number of tissues, such as the speen, the bone marrow, the liver, the adrenal glands. One that's a particular concern for me is it accumulates at quite high concentrations in the ovaries";
- "We thought the spike protein was a great target antigen we never knew the spike protein itself was a toxin and was a pathogenic protein. So by vaccinating people, we are inadvertently inoculating them with a toxin and some people, this gets into circulation and when that happens, in some people, it can cause damage, especially to the cardiovascular system."

Based on my review of this literature and data, my opinion is that Dr. Bridle's statements in the On Point with Alex Pierson podcast were not based in the evidence at the time, nor have they proven themselves to be accurate or data-based in retrospect.

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46. Shimabukuro, T.T., et al., *Safety monitoring in the Vaccine Adverse Event Reporting System (VAERS).* Vaccine, 2015. **33**(36): p. 4398-405.
47. Bettinger, J.A., et al., *2017/18 and 2018/19 seasonal influenza vaccine safety surveillance, Canadian National Vaccine Safety (CANVAS) Network.* Euro Surveill, 2020. **25**(22).
48. Thomas, S.J., et al., *Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months.* N Engl J Med, 2021. **385**(19): p. 1761-1773.
49. Piechotta, V., et al., *Safety and effectiveness of vaccines against COVID-19 in children aged 5-11 years: a systematic review and meta-analysis.* Lancet Child Adolesc Health, 2023. **7**(6): p. 379-391.

This is Exhibit "C" referred to in the Affidavit of Dr. Jason Kindrachuk in the City of Montreal, in the Province of Quebec, before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

KATHERINE R. COSTIN

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

ACKNOWLEDGMENT OF EXPERT'S DUTY

1. My name is Dr. Jason Kindrachuk. I live in the City of Winnipeg, in the Province of Manitoba.

2. I have been engaged by or on behalf of the Defendant, David Fisman, to provide evidence in relation to the above-noted court proceeding.

3. I acknowledge that it is my duty to provide evidence in relation to this proceeding as follows:

   (a) to provide opinion evidence that is fair, objective and non-partisan;

   (b) to provide opinion evidence that is related only to matters that are within my area of expertise; and

   (c) to provide such additional assistance as the Court may reasonably require, to determine a matter in issue.

4. I acknowledge that the duty referred to above prevails over any obligation which I may owe to any party by whom or on whose behalf I am engaged.

15-March-2024

Date _____

_____
Signature

NOTE: This form must be attached to any expert report under subrules 53.03(1) or (2) and any opinion evidence provided by an expert witness on a motion or application. RCP-E 53 (July 22, 2014)

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

PROCEEDING COMMENCED AT TORONTO

ACKNOWLEDGMENT OF EXPERT'S DUTY

LENCZNER SLAGHT LLP
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130 Adelaide Street West, Suite 2600
Toronto, ON M5H 3P5

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Email: jlilles@litigate.com
Katherine R. Costin (72173H)
Tel: (416) 865-3729
Email: kcostin@litigate.com

Lawyers for the Defendant,
David Fisman

Email for parties served:
Rocco Galati: rocco@idirect.com
Lynn Turnbull: lturnbull@curie.org

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

PROCEEDING COMMENCED AT TORONTO

AFFIDAVIT OF DR. JASON KINDRACHUK

LENCZNER SLAGHT LLP
Barristers
130 Adelaide Street West, Suite 2600
Toronto, ON M5H 3P5

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Tel: (416) 865-3552
Email: jlilles@litigate.com
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Lawyers for the Defendant,
David Fisman

Email for parties served:
Rocco Galati: rocco@idirect.com
Lynn Turnbull: lturnbull@curie.org

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

AFFIDAVIT OF DR. DAWN BOWDISH

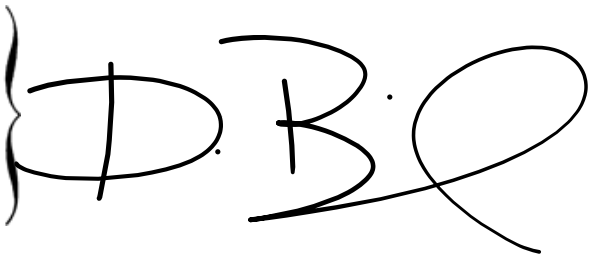
I, Dr. Dawn Bowdish, of the City of Hamilton, in the Province of Ontario, MAKE OATH AND SAY:

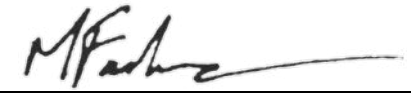
1. I have been asked by counsel to the defendant, Dr. David Fisman, to provide an expert opinion on the claims expressed on the COVID-19 vaccine safety and efficacy by Dr. Byram Bridle in June 2021 as part of this litigation**.** I am an immunologist, and, as such, have knowledge of the matters contained in this Affidavit. A copy of my CV is attached hereto as **Exhibit "A"**.

2. Attached hereto as **Exhibit "B"**, is a copy of my Expert Report dated March 14, 2024.

3. Attached hereto as **Exhibit "C"** is an executed copy of my Acknowledgment of Expert's Duty Form 53 dated March 15, 2024.

SWORN by Dr. Dawn Bowdish in the City of Hamilton, in the Province of Ontario, before me at the City of Toronto, in the Province of Ontario, on March 15, 2024 in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.





Commissioner for Taking Affidavits
(or as may be)

Mackenzie Daniel Frederick Faulkner, a Commissioner, etc., Province of Ontario, while a Student-at-Law. Expires April 6, 2025.

DR. DAWN BOWDISH

This is Exhibit "A" referred to in the Affidavit of Dr. Dawn Bowdish in the City of Hamilton, in the Province of Ontario before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

Mackenzie Daniel Frederick Faulkner, a Commissioner,
etc., Province of Ontario, while a Student-at-Law.
Expires April 6, 2025.

CURRICULUM VITAE

Dr. Dawn M. E. Bowdish
h-index = 60 (Google Scholar)

Business Address:
Department of Medicine
McMaster Immunology Research Centre
MG DeGroote Institute for Infectious Disease Research
McMaster University, MDCL 4020
1200 Main Street West
Hamilton ON L8N 3Z5
Tel: (+1) 905-525-9140, ext 22313
E-mail: bowdish@mcmaster.ca

Educational Background:

2005 – 2008 **Canadian Institute of Health Research Post-doctoral Fellow**
University of Oxford, Department of Pathology, Oxford U.K
Laboratory of Prof. Siamon Gordon
"Genetic and Functional Analysis of the scavenger receptor, MARCO"

2000 – 2005 **PhD. (Microbiology & Immunology)**
University of British Columbia, Dept. of Microbiology & Immunology Vancouver, Canada
Laboratory of Prof. R.E.W. Hancock
"Interactions of the human host defence peptide, LL-37 and the innate immune response"

1995 – 1999 **BSc. Honours (Microbiology)**
University of Guelph, Dept. of Microbiology, Guelph, Canada
Undergraduate project and summer studentship in the lab of Prof. J.S. Lam and supervised by Dr. Lori Burrows
"Genetics of the O-antigen of the O5 serogroup of *Pseudomonas aeruginosa*"

Honours:

2022	YWCA Hamilton & Halton – Woman of Distinction, Health & Recreation
2022	Meritorious Service Award – Lung Health Foundation
2020	Royal Society of Canada -College of New Scholars
2019-2024	Canada Research Chair in Aging & Immunity (Tier 2, renewed)
2019	McMaster Student's Union (MSU) Community Engagement Award
2018-2022	University Scholar
2016-2017	Breathe New Life Award – Top rated grant in the Ontario Thoracic Society/Ontario Lung Association's Grant in Aid program
2014-2019	Canada Research Chair in Aging & Immunity, Tier 2
2014	Best Teacher Award from the Department of Pathology & Molecular Medicine
2013	Nomination for the President's Award for Excellence in Graduate Student Supervision
2012	Ontario Lung Association-Pfizer Canada Research Award
2011	G. Jeanette Thorbecke New Investigator Award, Society of Leukocyte Biology
2010	ASPIRE, Pfizer Young Investigator Award for study of post-influenza pneumonia in the elderly
2006 – 2008	JMH Junior Research Fellowship awarded by Linacre College at the University of Oxford
2006	Cangene Gold Award, Awarded by the Canadian Society of Microbiology for the highest ranked PhD thesis in Microbiology
2005 – 2008	Post-doctoral Fellowship awarded by the Canadian Institutes of Health Research
2003 – 2005	Canada Graduate Scholarship awarded by the Canadian Institute of Health Research
February, 2005	Honorarium for having abstract chosen to present at the Innate Immunity in Human Disease Research Day: BC Research Institute for Children's & Women's Health
November, 2004	Travel Allowance awarded by the International Endotoxin Society 8th Biennial Conference of the International Endotoxin Society, Kyoto, Japan

Current Status at McMaster University:

August 2021-ongoing **Executive Director, Firestone Institute for Respiratory Health**
St. Joseph's Healthcare
McMaster University

January 2021-ongoing Professor, Tenured
Department of Medicine
MG DeGroote Institute for Infectious Disease Research
McMaster Research Immunology Centre
"Innate immunity and host-microbe interactions across the life course"

July 1, 2019- 2020 **Professor, Tenured**
Department of Pathology & Molecular Medicine
MG DeGroote Institute for Infectious Disease Research

McMaster Research Immunology Centre
"Innate immunity and host-microbe interactions across the life course"

July 1, 2014 – June 30, 2019 **Associate Professor, Tenured**
Department of Pathology & Molecular Medicine
MG DeGroote Institute for Infectious Disease Research
McMaster Research Immunology Centre
"The role of macrophage receptors in innate immunity and host defence"

February 1, 2009 – June 30, 2014 **Assistant Professor, Tenure-Track**
Department of Pathology & Molecular Medicine
MG DeGroote Institute for Infectious Disease Research
McMaster Research Immunology Centre
"The role of macrophage receptors in innate immunity and host defence"

Professional Organizations:

Canadian Society for Immunology
American Society for Immunology

Employment History:

Academic

August 2021 – ongoing: Executive Director, Firestone Institute for Respiratory Health
January 2021- ongoing: Professor, Department of Medicine, McMaster University, Hamilton, ON.
July 2019- Dec 2020: Professor, Department of Pathology & Molecular Medicine, McMaster University, Hamilton, ON.
July 2014- July 2019: Associate Professor, Department of Pathology & Molecular Medicine, McMaster University, Hamilton, ON.
February 1, 2009- June 30, 2014: Assistant Professor, Department of Pathology & Molecular Medicine, McMaster University, Hamilton, ON.

Scholarly & Professional Activities:

Professional development

Attended:

- Equitable Recruitment & Search/Selection Committee. January 22, 2021
- EnGender Workshop by The Executive Minds Inc. January – June 2018. This cross-faculty leadership training course addresses how change management, leadership styles and strategies for achieving professional goals.
- The Faculty of Health Sciences Leadership Management course 2016-2017
- The Emotionally Intelligent Manager: Ontario Hospital Association. November 25-27, 2015.

Completed:

- "R Basics – R programming Language Introduction" online course. Udemy July, 2016

Journal Referee (ad hoc)

Cell Host & Microbe, Journal of Infectious Disease, PLoS Pathogens, Journal of Leukocyte Biology, Molecular Therapeutics, Mucosal Immunology, Frontiers in Immunology, American Journal of Respiratory Cell and Molecular Biology, American Journal of Respiratory and Critical Care Medicine, Journal of Immunology, PLoS ONE, Journal of Innate Immunity, Journal of Royal

Society Interface, Journal of Inflammation, Infection & Immunity, American Journal of Physiology, Molecular Autism and others

Journal Editor

- Immunity & Ageing Journal (2019-ongoing)

Guest Editor

- Guest editor for special issue entitled "Bioinformatics for Immunologists" of Frontiers in Immunology (2014-2015)

External Grant Reviews (Local)

- Research Institute of St. Joseph's Hamilton Research Awards (2021, 2022, 2023)

External Grant Reviews (Canadian)

-Canada Research Chair (ongoing, ad hoc)
-NSERC Discovery Grant Competition (Jan 2011, 2013, 2016, 2017, 2018, 2019,2020, 2023)
-CIHR Microbiology & Infectious Disease Panel

- Chair November 2022,
- Scientific Officer December 2017, March 2018, December 2019, August 2020,
- Reviewer for Project/Operating Grant program May 2013, 2023

-Respiratory Research Committee of Saskatchewan (2021)
-CIHR College of Reviewers (2017-ongoing)
-The Lung Association (Basic Science panel, March 2014, 2018, 2019)
-Banting Research Foundation (2016-2019)
-CIHR (Biomedical Sciences, Institute of Aging) (2014)
-Multiple Sclerosis Society of Canada (Operating Grant competition, Jan 2011 & 2013)

External Grant Reviews (International)

-Yale Pepper Geroscience New Investigator awards (ad hoc reviewer 2023)
-Hemolz Distinguished Professorship, Germany 2022
-NIH Program Grant – PO3, 2021
-Reta Lila Weston Trust (Microbiome & Brain Health grants, 2020)
-Irish Science Foundation (Ireland) (2016, 2017, 2019,2020)
-Rosetrees Trust (2020)
-Dutch Research Council (2020)
-Biotechnology & Biological Sciences Research Council (UK) (2014, 2015, 2018)
-NIH – Project Program Grant (2017, 2018)
-Medical Research Councils UK (2016, 2017)
-Scottish MRC – MIDAS-RTI (2017)
-Fondazione Cariplo, Italy (2016)
-ANR (Agence Nationale du Récherche), France (2012, 2016)
-Israeli Science Foundation (2014)
-Asthma UK (2012)

Contributions to Scientific Societies

<u>Board of Directors, Lung Health Foundation (formerly Ontario Lung Association)</u> (June 2015-ongoing)
Major initiatives include;

i) Development of the "National Lung Health Agenda", which is a plan to create a federal action plan to improve lung health in Canada
ii) Built new pillars of research and investment to align with changing demographics of lung disease and changes in philanthropic giving.

iii) Oversaw the transition from a federated association to the Lung Health Foundation, including creating the Lung Health Foundation Advisory Board.
iv) participating in the "Breathing as One" campaign, designed to increase research funding for lung health by 10-12 million in the next 5 years. (2014-present)
v) Advocating for Bill 71: Lung Health Act, a private members bill to create a lung health action plan for Ontario (2014-present).
vi) spokesperson for International Pneumonia Day 2017, 2018 and the vaccination campaign for pneumococcal pneumonia.
https://www.facebook.com/OntarioLungAssociation/videos/596521740766963/
vii) Strategic Directions Committee (2016-ongoing): Oversees strategic investments, develops and approves new initiatives, assesses changing demographics of lung disease to best target initiatives

Scientific Advisory Board – British Society for Research on Ageing (2023-ongoing)

Organizing committee for the 2020 International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD), Toronto, Ontario. (postponed to 2022 due to COVID-19)

Organizing committee for the International Infection, Immunity and Inflammation Conference (I4C)
May 14-15, 2019, Vancouver, BC Canada

Discussion Leader at the 2019 Gordon conference on Antimicrobial peptides (Barga, Italy) and 2020 Gordon Conference on Acute Respiratory Infections (Galveston, Texas)

Vice-chair of the 2016 Gordon conference on Acute Respiratory Infections (Galveston, Texas)
co-Chair of the 2018 Gordon conference on Acute Respiratory Infections (Ventura, California)

Scientific Advisory Board, "Exploiting plant-made vaccines to protect the elderly against respiratory viruses"
Génome Québec / le Ministère de l'Économie, de la Science et de l'Innovation (2017-2019)

Executive committee of the Ontario Thoracic Society (2018-2022)

Consulting

COVID-19 Strategic Consulting Group (SCG)- AstraZeneca Global (2022-ongoing)

Ontario Immunization Advisory Group, December 2021.
-shared data on vaccine efficacy in long-term care and retirement home residents and made recommendations for 4th doses to assist with the omicron wave. These recommendations were shared with the Public Health Agency of Canada and implemented in January 2022. "COVID-19 Vaccine Recommendations in the Context of the Omicron Variant, 23 December 2021"
https://cm.publichealthontario.ca/-

/media/documents/ncov/vaccines/2021/12/covid19-vaccine-recommendations-omicron-variant.pdf?sc_lang=en

Pfizer Advisors and Vaccine Experts (PAVE), 2019
Qu-Biologics (Vancouver, Canada) 2013-2017
Miami Mice (Toronto, Canada) 2014-2015

Expert Witness Activities

Nabil Ben Naoum vs. L'honorable Maxime Bernier, Canada. *Wrote a report and was cross examined*. June-September, 2022
David Lavergne-Poitras vs. PMG Technologies Inc., Canada, Montreal, QC. *Wrote a report and was cross examined*. May-July 2022
Syndicat des Metallos S.L., 2008, 9599, 2004, 9344, 9554, 1976, 9449, 9519, 5778, 9996 et als. vs. Procureur General du Canada, Canada, Montreal, QC. *Wrote a report*. March-May 2022

Administrative Responsibilities:
Committee Membership

University

- Canada's Global Nexus for Pandemic Preparedness and Biologic Threats – Task Force Chair (2021-ongoing)
- FHS Equity, Diversity & Inclusion (2019-ongoing)
- Braley Centre for Antimicrobial Resistance Executive Committee (2019-ongoing)
- Assistant Director IIDR - Training(2019-2021)
    - Major contributions include developing the Braley Fellows program in antimicrobial resistance
- IIDR Executive Committee (2015- ongoing)
- CFI Infrastructure internal review committee (2016)
- Animal Research Ethics Board member (2014-2017)
- Focus Group on Identification and Support of Emerging Leaders at the Assistant and Associate Professor Levels (2014-2017)
- Graduate Council Scholarships Member: Rank scholarships for OGS, NSERC, CIHR (Oct 2010 – Aug 2013)

Department

- Institute for Infectious Disease Research Symposium/Trainee Day Organizing Committee – 2010, 2018, 2019
- Tenure & Promotion Committee – Pathology & Molecular Medicine, 2017-ongoing.
- MIRC communications committee 2013-ongoing
    - responsible for website design, content and social media activities
- Institute for Infectious Disease Research Communications Committee Aug 2013-Aug 2014
- Organizer of Trainee Poster Presentations and Awards: Michael G. DeGroote Institute for Infectious Disease Research Opening Symposium (October 24th, 2009)

Faculty

- Chemistry Faculty Search Committee Oct-Dec 2018
- Judge for the FHS Post-doctoral Fellow Association Annual Presentation day (2018)
- Infection & Immunity Area Co-ordinator for the Medical Sciences Graduate Program (2012-2013).

- FHS Research Plenary Award Selection Committee: Selected graduate winners of awards presented at the Inaugural Faculty of Health Sciences Research Plenary (May 4th, 2010)
- Judge – Poster session: 1st Annual FHS Postdoctoral Research Day (June, 3rd, 2010)
- Judge – Student Award Symposium: Canadian Society of Microbiology 2010 Annual Conference (June 2010)

Areas of Interest:

Research: COVID-19 infections and vaccinations, immunosenescence, macrophage biology, macrophage receptor expression, scavenger receptor and phagocytic receptor function and signalling, aging and immunity, innate immunity & host defence, bacterial colonization and infections (e.g. Streptococcus pneumoniae), animal models of pneumonia and post-influenza pneumonia, microbiome of the upper respiratory tract, pathogens & commensals of the upper respiratory tract

Teaching: immunology, innate immunity, host defence, immunosenescence, host-pathogen interactions

Courses Taught:	**Course Title**
Undergraduate	
NEXUS2A03	Impact of Infectious Disease on Individuals and Society -Lecturer (2 lectures, 2024).
HTH SCI 4II3	Advanced Immunology (Jan 2011-2019-ongoing) – Course co-ordinator (until 2020) & lecturer (ongoing, 3-4 lecturers/year)
HTH SCI 3I03	Immunology (Sept 2015-ongoing, 1 lecture/semester)
HTH SCI 1DT3	Introduction to Immunology (Jan 2014-ongoing, 2 lectures/semester)
BDC4B03	Commercialization planning (2017W)
BDC3A03	Road to Biomedical Discovery (November 2016, 2017)
HTH SCI 4DM3	Demystifying Medicine (Sept 2013-Dec 2013)
Graduate Lectures:	
MS 722	Health Science Communication (1 lecture per term 2022-ongoing)
MS 715	Advanced Immunobiology (1 lecture per term 2010-2019)
BBS 771	Immunometabolism (1 lecture per term 2016-2019)
Graduate Courses:	
MS 730	Antimicrobial Resistance from principles to practice (2019 -2021)
MS 799	Independent Study in Medical Sciences (2010 & 2012)

External teaching

MICR 4010	Bacterial Pathogenesis (1 lecture), University of Guelph (January 2014, February 2015, and February 2016)

Supervisorships:
(For a complete list of awards and distinctions received by trainees see www.bowdish.ca/people)

MSc (in progress)
Lachlan MacLean (Medical Sciences) 2023-ongoing "Targeting macrophages to ameliorate pulmonary

		fibrosis"
Alice Caldwell (Medical Sciences)	2023-ongoing	"Understanding how aging gut physiology impacts age-associated inflammation."

MSc (completed)

Dominika Boron (Medical Sciences)	2020-2022	"Features of the upper respiratory tract microbiome associated with infection"

(Ontario Graduate Scholarship 2020/21, Award for best short talk at the Medical Sciences Graduate Program Virtual Research Day March 2021, CIHR Canada Graduate Scholarship 2021-2022, Selected talk for the Mildred Gulliver award competition – IIDR trainee day 2021)

Anastasia Chouvalov (Medical Sciences)	2019-2021	"Early life adversity alters the aging trajectory"
Diana Mirceta (U. Copenhagen)	2020-2021	"Identifying members of the upper respiratory microbiota with anti-pneumococcal properties"
Erica DeJong	2018-2020	"The microbiota and unhealthy aging"

(Ontario Graduate Scholarship 2019/20 Certificate of Excellence from the Summer Program in Aging, CIHR 2019)

Grace Teskey	2016-2018	"Peripheral immunophenotype is changed during autism spectrum disorder"
Aveshni Naidoo	2013-2016	"Age-associated inflammation impairs the anti-bacterial activity of macrophages"
Netusha Thevaranjan	2014-2016	"Age related changes in the microbiome of the URT predispose the elderly to *S. pneumoniae* infection"
Nick Yap (co-supervised with Brian Golding)	2014 – 2016	"Phylogenetic approaches to characterize the evolution of pattern recognition receptors"
Fiona Whelan	2010 - 2012	"Evolution of the class A scavenger receptors"
Zhongyuan Tu	2010 - 2012	"Discovery of novel signalling motifs in the cytoplasmic domain of MARCO"
Mariliis Kroos (co-supervised with Prof. Siamon Gordon, University of Oxford	2007- 2008	"The role of the cytoplasmic domain of MARCO in adhesion and motility"

PhD (in progress)

Sofya Ermolina (Medical Sciences)	2021-ongoing	"The role of the microbiome in unhealthy aging"

(Department of Medicine Graduate Student Incentive Program Award 2022/23, MIRA Graduate Student Professional Development 2022)

Nancy ElChaar	2021-ongoing	"Identifying anti-pneumococcal features of the Upper Respiratory Tract"

Christian Bellissimo 2022-ongoing "Characterizing placentation during metabolic stress"
(Ontario Graduate Scholarship 2017, Canada Graduate Scholarship-MSC 2018, Canada Graduate Scholarhsip
Doctoral 2019, Best Poster Presentation in Placental Biology at the Canadian National Perinatal ResearchMeeting 2019)

Jenna Benoit (Medical Sciences) 2021-ongoing "COVID-19 vaccination efficacy in patients on immunomodulatory drugs"
(Ontario Graduate Scholarship 2022, Firestone Institute for Respiratory Health Travel grant to present selected abstract (oral presentation) at the Canadian Society of Immunology conference 2022, Poster award at the Canadian Society of Immunology 2022, McMaster Global Experiences Travel Award present chosen abstract at FOCIS 2022, Second place for best PhD presentation at the 2022 Perey Symposium)

Kevin Zhao 2021-ongoing "TNF impairs macrophage killing of *Streptococcus pneumoniae"*
(Medical Sciences, MD/PhD program)
(Winner of the McMaster Institute of Research on Aging Pitch Your Project "People's Choice Awards" 2021, MIRA-IIDR PhD scholarship 2022, Ontario Graduate Scholarship 2022, First place PhD short presentation at the 8th Annual Perey Symposium 2022, CIHR-CGS-D-2023-2026)

PhD (completed)

Elnur Shayhidin (Medical Sciences) 2019-2021 "TNF impairs macrophage killing of *Streptococcus* pneumoniae"
(withdrawn due to COVID, currently industry)

Jessica Breznik(Medical Sciences) 2016-2020 "Peripheral Monocytes and Intestinal Macrophages during Chronic Inflammation"
Current position: PDF (McMaster, Bowdish lab)

Sara Makaremi (Biomedical Engineering)2014-2020 "Structural requirements for macrophage adhesion"
(co-supervised with Jose Moran-Mirabal)
Current position: PDF (US)

Pat Schenck (Biochemistry) 2014-2019 "The composition of the airway microbiota influences infection risk"
(co-supervised with Mike Surette)
Current position: Scientific Advisor, Research Foundation

Dessi Loukov (Medical Sciences) 2014-2018 "Age-associated inflammation drives macrophage dysfunction and susceptibility to pneumococcal infection"
Current position: Medical Science Liaison

Kyle Novakowski (Medical Sciences) 2012- 2017 "The importance of the scavenger receptor cysteine rich domain of MARCO in bacterial recognition & signalling"
Current position: Industry (Canada)

Fan Fei (Chemistry) 2013-2015 "Metabolomic analysis of the inflammatory response
of Current position: Industry (US) macrophages from young and old mice"

Mike Dorrington (Medical Sciences) 2010 – 2015 "The role of MARCO in control of pneumococcal

Current position: Scientific Advisor, Ontario Genomics Institute		colonization of the nasopharynx"
Alicja Puchta (Medical Sciences) Current position: Lawyer	2010- 2014	"Age associated macrophage dysfunction contributes to susceptibility to pneumonia"

Post-doctoral (in progress)

Dr. Kate Kennedy	2021-ongoing	"Maternal-microbiome relationships in pregnancy and impact on offspring intestinal development"

(Award for talk at the 2022 Canadian National Perinatal Research Meeting, Molly Towell Perinatal Research Foundation 2022)

Dr. Jessica Breznik	2020-ongoing	"Immunologic predictors of COVID-19 risk in long term care"

(MIRA fellowship 2021-2022, Best PDF poster- IIDR Trainee Day 2021, Poster award in Maternal & Fetal Medicine at the 2022 Canadian National Perinatal Research Meeting)

Post-doctoral (completed)

Dr. Candice Quin	2020-2023	"Age-associated inflammation alters myelopoiesis"

(CIHR Fellowship 2020-2023, Mildred Gulliver Post-doctoral fellow award 2021, Best poster at the IIDR trainee day 2020, Winner of the Perey Symposium postdoctoral fellowship award for best oral presentation 2022)
Current position: Faculty at the University of Aberdeen

Dr. Tatianne Ribeiro (co-supervised with Dr. Deborah Sloboda, MIRA & M. G. DeGroote Fellowship recipient)	2019-2021	"Early life adversity alters immune function"
Dr. Allison Kennedy (co-supervised with Dr. Doug Boreham) Current position: Research Associate (McMaster)	2017-2020	"Peripheral immune changes in prostate cancer patients undergoing low dose radiation"
Dr. Janine Strehmel Current position: Industry (Toronto)	2016-2019	"Mining the URT microbiota for novel antimicrobials"
Dr. Cedoljub Bundalovic-Torma (co-supervised with Dr. Mike Surette) Current position: PDF – U of Toronto	2018-2019	"Age-related changes to the upper respiratory tract microbiota"
Dr. Christian Schulz (DFG fellowship recipient) Current position: Industry (Germany)	2016-2018	"Host-microbiome interactions influence colonization and infection with *S. pneumoniae"*
Dr. Chris Verschoor Current position: Faculty, HSNRI	2010- 2016	"Age-associated myeloid cell immunosenescence contributes to susceptibility of the elderly to *Streptococcus pneumoniae*"
Dr. Preethi Jayanth	2011 – 2013	Co-supervised with Dr. Mike Surette

Current position: Technician, U of Guelph		"Mechanisms of host immune evasion by the *Streptococcus milleri* group"
Dr. Peter Pelka Current position: Faculty, U of Manitoba	2011 – 2012	"Host immune responses to the microbiota of the upper respiratory tract"

Undergraduate students

Amanda Densil	09/2023-04/2024	4th year Project Student
Molly Health	09/2023-04/2024	4th year Project Student
	05/2023-09/2023	NSERC USRA summer student
Aunika Venables	09/2023-04/2024	iSci 4th Project Student
	01/2023-04/2023	iSci 3rd year project student
Catharine Andary	09/2022 -ongoing	Medical student
Minuki Wickramasuriya	05/2022-08/2022	Co-op student
Alexander Georgiou	01/2022-04/2022	Co-op student
Clare Edwards	09/2021-04/2022	4th year thesis student
	01/2021-08/2021	Co-op student
Ansha Suleman	09/2020-04/2021	4th year thesis student
Kevin Dai	09/2020-04/2021	4th year thesis student
Christina Cerson	09/2020-04/2021	4th year thesis student (co-supervised with Dr. Jose Moran-Mirabal)
	05/2019-08/2019	NSERC Summer student (co-supervised with Dr. Jose Moran-Mirabal)
Alisa Nykolayeva	06/2020-12/2020	Co-op student
Maiura Maralitharan	09/2019-04/2020	4th year thesis student
Kate Miyasaki	09/2019-04/2020	3rd year thesis student
Sonia Igboanugo	08/2018-04/2019	BDC 4th year student
Danny Ma	05/2018-04/2019	NSERC USRA (Summer), BHSc thesis (Fall/Winter)
Mina Sadeghi	05/2018-04/2019	Biopharm thesis (Summer), Co-op (Fall/Winter)
Judjina Thevaranjah	01/2018-12/2018	Co-op student (Winter/Summer semester), Thesis (Fall)
Melodie Na-Yoon Kim	05/2017-09/2018	4th year BHSc student HTH SCI
	08/2018-04/2019	4th year BHSc student HTH SCI
Joseph Chong	09/2017-04/2018	4th year Biochemistry student
Dhanyasri Maddiboina	09/2017-04/2018	iSci- 4th year thesis student
Joseph Chong	09/2017-04/2018	4th year Biochemistry student
	09/2016-04/2017	3rd year Biochemistry student
Sureka Pavalagantharajah	09/2016-04/2017	4th year BHSc
Dhanyasri Maddiboina	09/2016-04/2017	iSci-3rd year thesis student
Jason Fan	09/2016-04/2017	4th year BHSc
	09/2015-04/2016	3rd year BHSc HTHSCI3H03
Mohammad Malik	05/2016-09/2016	Biotechnology co-op student
Melodie Na-Yoon Kim	05/2016-09/2016	3rd year BHSc student HTH SCI 3H03
Jenny Sun	01/2016-08/2016	Biology & Pharmacology co-op student
Justin Boyle	09/2015-04/2016	Chemistry Senior Thesis student (co-supervised with Dr. José Moran-Mirabal)
Thilini Delungahawatta	09/2015-04/2016	Biology Senior Thesis student (Biol 4C09, co-supervised with David Rollo)
Vikash Chawla	09/2015-04/2016	Biology Senior Thesis student (Biol 4C09)

Alejandra Lagos	09/2015-04/2016	Biology Senior Thesis student (Biol 4C09)
Mohamad Malik	01/2015-08/2015	Biotechnology co-op student
Alan Zhou	01/2015-04/2015	iSci-3rd year thesis student
Ben Su	09/2014-04/2015	Academic advisor for SCIENCE 3EX6 Applied Placement
Melissa Ling	01/2014-04/2014	iSci-3rd year thesis student
	09/2014-04/2015	iSci- 4th year thesis student
Dessi Loukov	01/2014-04/2014	4th year thesis student
Philip Lauman	01/2014-04/2014	iSci-3rd year thesis student
Jessica Wallace	09/2013-04/2014	Biology 4th year thesis student
Robert Valencia	09/2013-04/2014	Lab Co-ordinator
James Han	05/2013-04/2014	iSci- 4th year thesis student (ISCI 4A12)
Netusha Thevaranjan	05/2013-04/2014	Undergraduate Technician, Summer student, Biology-4th year thesis student (Life Sci 4C09)
Charles Yin	09/2013-04/2014	4th year thesis student (ISCI 4A12)
	05/2012-08/2012	iSci- 3rd year thesis student
Prashant Kalvapalle	05/2013-08/2013	MITACS summer studentship recipient
Samathy Balachandran	01/2013-04/2013	Pharmacology – 4th year thesis student
Peter Mu	08/2011-04/2013	Undergraduate Lab Manager
Keith Lee	05/2012-04/2013	BHSc- 4th year thesis student
Jaskiran Nanda	05/2011-08/2011	Biology -Summer research student
Michael Danesi	05/2011-08/2011	Biology & Psychology –Summer Research
Sarah Chauvin	06/2010-04/2011	Biochemistry- 3rd year project & 4th year thesis
Harikesh Wong	07/2009-04/2010	Biochemistry- 4th year thesis
Alex Jiang	06/2009-04/2012	BHSc- 3rd & 4th year thesis
Zhongyuan Tu	05/2009-08/2010	Biochemistry -4th year thesis

Supervisory Committees (in progress)

Kayla Zhang	2024-ongoing	MSc, Medical Sciences (supervisor, Dr. M. Mukherjee)
Elizabeth Ball	2023-ongoing	PhD, Medical Sciences (supervisor, Dr. J. Hirota)
Angela Schmidt	2023-ongoing	MSc, Medical Sciences (supervisor, Dr. J. Schertzer)
Sara Deir	2023-ongoing	MSc, Biomedical Engineering (supervisor, Dr. B. Zhang)
Quan Zhou	2023-ongoing	PhD, Medical Sciences (supervisor, Dr. M. Kolb)
Yona Tugg	2022-ongoing	PhD, Medical Sciences (supervisor, Dr. Matt Miller)
Nuzhat Rahman	2021-ongoing	PhD, Medical Sciences (supervisor, Dr. Charu Kausic)
Seyed Saeid Tabatabaei	2021-ongoing	PhD, University of Guelph, Animal Sciences (supervisor – Dr. Caswell)
Kyle Jackson	2021-ongoing	PhD, Chemical Engineering (supervisor – Dr. Housseini-Doust)
Amber Hann	2020-ongoing	PhD, Biochemistry (supervisor – Dr. Verdú)
Anita Singh	2020-ongoing	MSc, Biochemistry (supervisor – Dr. Schertzer)
Marie-Ange Massicotte	2020-ongoing	PhD, Biochemistry (supervisor – Dr. Coombes)
Summer Cho	2020-ongoing	PhD, Biochemistry (supervisor – Dr. Wright-MacNeil)
Shawna Thompson	2016-ongoing	PhD, Psychiatry (supervisor – Dr. Foster)
Alex Qian	2016-ongoing	PhD, Biochemistry (supervisor – Dr. Trigatti)

Supervisory Committees (completed)

Zeina Saayfane	2022-withdrew 2023	MSc, Medical Sciences (supervisor Dr. Hawke)
Anurag Bhalla	2020-2022	MSc, Medical Sciences (supervisor – Dr. Nair)
Michael Huang	2020-withdrew 2022	MSc, Biochemistry (supervisor – Dr. Schertzer)

Sabah Haq	2018-2022	PhD, Medical Sciences (supervisor – Dr. Khan)
Christian Bellissimo	2018-2022	PhD, Biochemistry (supervisor – Dr. Sloboda)
Sarvatit Patel	2018-2022	MSc, Chemical Biology (supervisor – Dr. Werstuck)
Josh McGrath	2016-2021	PhD, Pathology (supervisor – Dr. Stampfli)
Katherine Kennedy	2015-2021	PhD, Biochemistry (supervisor – Dr. Sloboda)
Alexandra Parco	2020-2021	MSc, Biochemistry (supervisor – Dr. Coombes)
Kaylyn Bacchiocchi	2020-2021	MSc, Biochemistry (supervisor – Dr. Bramson)
Caressa Tsai	2017-2021	PhD, Biochemistry (supervisor – Dr. Coombes)
Elizabeth Chau	2016-2021	PhD, Biochemistry (supervisor – Dr. Coombes)
Mila Bjelica	2019-2021	MSc, Kinesiology (supervisor – Dr. Obeid)
Marcus Rose	2019-2020	PhD, Physics & Astronomy (supervisors – Dr. Fradin & Dr. Moran-Mirabal)
Inna Ushcatz	2019-2020	MSc, Medicine (supervisor – Dr. Obeid)
Blerina Kadiu	2019-2020	PhD, Biochemistry (supervisor – Dr. Surette)
David Hare	2013-2020	PhD, Pathology (supervisor – Dr. Mossman)
Ken Mwawasi	2015-2020	PhD, Pathology (supervisor – Dr. Bramson)
Trevor Lau	2015-2020	PhD, Biochemistry (supervisor – Dr. Schertzer)
Gayatri Nair	2018-2019	MSc, Medical Sciences (supervisor – Dr. Surette)
Alexis Bullock	2018-2019	MSc, Kinesiology (supervisor – Dr. Heisz)
Maryam Vaseghi-Shanjani	2017-2019	MSc, Medical Sciences (supervisor – Dr. Xing)
Bushra Ilyas	2014-2019	PhD, Biochemistry (supervisor – Dr. Coombes)
Jeff Lam	2017-2019	MSc, Pathology (supervisor – Dr. Kaushic)
Ophélie Quillier	2016-2018	Biochemistry (supervisor – Dr. Surette)
Vi Dang	2014-2018	PhD, Biochemistry (supervisor – Dr. Werstuck)
Jenna Dowhaniuk	2015-2017	MSc, Medicine (supervisor – Dr. Ratcliffe)
Aric Huang	2016-2017	MSc, Biochemistry (supervisor – Dr. Werstuck)
Sohail Mahmood	2014-2017	MSc, Pathology (supervisor -Dr. Ask)
Emily Paolucci	2016-2017	MSc, Kinesiology (supervisor – Dr. Heisz)
Brian Tuinema	2014-2017	PhD, Biochemistry (supervisor – Dr. Coombes)
Sara Dizzel	2015-2017	MSc, Pathology (supervisor – Dr. Kaushic)
Phil Staibano	2015-2016	MSc, Medicine (supervisor – Dr. Nazi)
Michelle Mendoça	2011 – 2016	PhD, Biochemistry (supervisor – Dr. Surette)
Stacey Muise	2015 – 2016	MSc, Radiation Medicine (supervisor – Dr. Boreham)
Jessica Wallace	2015 – 2016	MSc, Biochemistry (supervisor – Dr. Sloboda)
Jane McBride	2015 – 2016	MSc, Biochemistry (supervisor – Dr. Schertzer)
Ashley Beaulieu	2015 – 2016	MSc, Pathology (supervisor – Dr. Stampfli)
Sarah Karampatos	2015 – 2016	MSc, Rehabilitation Science (supervisor – Dr. Maly)
Pamela Shen	2011 – 2016	PhD, Pathology (supervisor – Dr. Stampfli)
Karissa Giraldi	2013 – 2015	MSc, Biochemistry (supervisor - Dr. Surette)
Tatianna Wong	2013 – 2015	MSc, Biochemistry (supervisor – Dr. Gilberger)
Ken Mwasi	2013 – 2014	MSc, Pathology (supervisor – Dr. Mahoney)
Vi Dang	2013 – 2014	MSc, Chemistry (supervisor – Dr. McCarry/Britz-McKibbin)
Alexandra Ruyter	2011 – 2013	MSc, Pathology (supervisor – Dr. Mahoney)
Julie Kaiser	2011 – 2013	MSc, Biochemistry (supervisor – Dr. Surette)
Tamara Krneta	2010 – 2012	MSc, Pathology (supervisor – Dr. Ashkar)
Elena Pretus	2010 – 2012	MSc, Pathology (supervisor – Dr. Stampfli)
Lauren Waller	2010 – 2012	MSc, Biochemistry (supervisor – Dr. Coombes)
Elspeth Smith	2010 – 2012	MSc, Pathology (supervisor – Dr. Lichty)

Josip Marcinko	2009 – 2011	MSc, Pathology (supervisor – Dr. Jordana)
Devanghi Mehta	2009 – 2011	MSc, Pathology (supervisor – Dr. Mossman)

External Examiner - **Comprehensive Examiner**

Mikela Eng	September 2020	McMaster University
Danya Thayaparan	April 2019	McMaster University
Kyle Flannigan	March 2014	McMaster University
Varun Anipindi	May 2012	McMaster University
Bethany Heinrick	July 2010	McMaster University
Dannie Bernard	June 2009	McMaster University

Comprehensive Exam advisor

Quan Zhou	February 2024	McMaster University
Alex Qian	May 2019	McMaster University
Ken Mwawasi	June 2016	McMaster University
Pamela Shen	May 2013	McMaster University

External Examiner- PhD Transfer

Sophie Pozanski (Pathology)	May 2018	McMaster University
Daphne Lamarchée(Biochemistry)	Jun 2015	McMaster University
Uyen Nguyen (Biochemistry)	Apr 2013	McMaster University
Brian Tuinema (Biochemistry)	Feb 2013	McMaster University
Leticia Gonzalez Jara (Biochemistry)	June 2012	McMaster University
Carly Horvath (Pathology)	July 2010	McMaster University
Afia Aziz Ur Rehman(Medicine)	Dec 2010	McMaster University

Chair- PhD Transfer

John-Paul Oliveria	June 2013	McMaster University

Chair- PhD Defence

Hannah Stacey	April 2023	McMaster University
Dharneya Thayaparan	June 2021	McMaster University
Victor Ferriera	November 2014	McMaster University

External Examiner- MSc defense

Amy Moorhead	September 2020	McMaster University
Andrea Monjo	December 2016	Sir Wilfred Laurier University
Sarah Poynter	August 2013	Sir Wilfred Laurier University
Joshua Kong	September 2013	McMaster University
Elena Pretus	August 2012	McMaster University
Elishka Pek	July 2009	McMaster University
Aman Thatte	December 2009	McMaster University

External Examiner- PhD defense

Saoirse Benson	June 2023	Flinders University, Australia
Jenny Nguyen	May 2023	University of Calgary
Melanie Girard	December 2022	University of Toronto
Nadia Milad	September 2022	Sherbrooke University
Sasha Doodnauth	October 2020	University of Toronto

Kayla Campbell	August 2020	Brown University
Ravi Holani	March 2020	University of Calgary
Elena Mitsi	December 2019	Liverpool School of Tropical Health, UK
Sara Roggensack	November 2019	Tufts University, US
Victora Hippolito	August 2019	University of Toronto
Angela Li	February 2019	University of Toronto
Danielle Twum	November 2018	Roswell Park Cancer Institute, US
Breanna Hodgins	October 2018	McGill University
Jieun Kim	February 2018	University of Toronto
Alyssa Cull	February 2017	Queen's University
Otto Strauss	May 2016	University of Auckland, New Zealand
Emma de Jong	December 2015	Murdoch University, Australia.
Amra Saric	December 2015	Ryerson University
Cameron Stuart McAlpine	June 2015	McMaster University
Paula Beaumont	September 2014	University of Edinburgh, Scotland, UK
Aswin Hari	October 2013	University of Calgary
Kenneth Chalcraft	March 2013	McMaster University
Katherine J. Kasper	January 2013	University of Western Ontario
Amal Al-Gawari	June 2012	McMaster University

Other contributions to teaching

Supervision of foreign/exchange students

Lara Jaëske	Sep 2019-Nov 2019	University of Rostock, Germany
Andrea Kellner	Sept 2015-March 2016	University of Jena, Germany
Prashant Kalvapalie	May 2013-Aug 2013	MITACS summer exchange student

Grade School

Dundas Central – Grad 5 class "Food microbiology" (3 science classes)	March-April 2019
Dundas Central – Grade 4 class "Dirt Microbiology" (2 science classes)	March 2017, Oct 2018
Dundas Central-Grade 1 class "Good germs/bad germs" (2 science classes)	January 2014

High School students

1. Anika Gupta (Sept 2017-May2018 & Sept 2018-May2019), High School Student, Bay Area Science and Engineering Fair project
2. Kazi Akther (August 2014), High School Student, winner of the IIDR internship for the Bay Area Science & Engineering Fair
3. Priynka Dhillon (August 2014), High School Student, winner of the IIDR internship for the Bay Area Science & Engineering Fair
4. Catharine Bowman, High School Student, performed a science fair project for the Bay Area Science and Engineering Fair (Jan- Mar 2014).
5. Eisha Ahmed, High School Student, competing in the International Science Fair (Apr -Aug 2013), supervised preparation for the International Science fair & hosted with Dr. Mike Surette for her receipt of the IIDR Summer Student Internship
6. Leonard Rivet, High School Student, member of Youth Engaged in Science (YES) Mentorship program (Sept 2012-June 2013, with Dr. Alba Guarné)
7. Jason Fan, High School Student, IIDR internship winner (May- Aug 2012)
8. Julia Lee, High School Student, member of Youth Engaged in Science (YES) Mentorship program (Oct 2009-Mar 2010)

Other

- Mohammed Malik (Jan 2015-Aug 2015), Co-op student, Biotechnology Program
- Judge for Trainee presentations at the 1st Annual Perey Symposium (June 2014) and member of awards committee
- Dessi Loukov, Co-op student, industrial student with Qu Biologics (May –Dec 2013)

Research Funding:

Currently Held:

2023-09-14 – 2028-09-14	Unraveling the role of IgA during respiratory virus infections Infection and Immunity Canadian Istitutes of Health Research PI: M. Loeb Collaborator: D. Bowdish	$914,175 ($0 to Bowdish)
2023-11-01 – 2026-11-31	Uncovering the role of chronic inflammation and serious respiratory infections in brain aging and cognitive impairment Operating Grant: Mechanisms in Brain Aging and Dementia PIs: D. Bowdish & C. Verschoor (HSRNI) coPIs: A. Jones, M. Chong, A. Costa, K. Choe (McMaster), J. Kwong (UofT), M. Tremblay (Victoria), C. Quin (Aberdeen)	750,000 (250K/yr)
2023-04-1- 2028-03-31	Uncovering the role of the gut microbiome in unhealthy aging and frailty Canadian Institutes of Health Research PI: D. Bowdish Co-Investigator: M. Surette	$856,800 ($171,360/year)
2023-04-01- 2028-03-31	Reprogramming lung macrophages using inhalable immunomodulatory microparticles for the treatment of lung fibrosis Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Horizons PI: T. Hoare Collaborators: D. Bowdish, M. Kolb, R. Wylie	$500,000 ($25,000/year to Bowdish)
2021-04-01- 2023-03-31	A living evidence approach to variants of concern (VOC) and COVID-19 vaccine effectiveness Canadian Institutes for Health Research (CIHR) PI: J Little Co-PI: A Iorio. Co-Is: M Begin, J Beyene, I Boutron, D Bowdish, M Brouwers, C Colijn, C Cooper, A Costa, D Coyle, J Curran, D Earn, K El Emam, L Griffith, J Grimshaw, J Heffernan, T Horsley, A Hsu, S Hughes, B Kagina, M Langlois, J Lavis, X Li, S O'Brien, A Pham-Huy, T Piggott, P Raina, M Rubini, M Smith, L Thabane, H Wang, V Welch, K Wilson, L Xu	$475,940 ($0 to Bowdish)
2021-04-01- 2023-03-31	Safety immUnogenicity of Covid-19 vaCcines in systEmic immunE mediated inflammatory Diseases (SUCCEED)	$3,131,216 (McMaster

	COVID-19 Immunity Task Force McGill: S Bernatsky, I Colmegna UHN/Sinai/Toronto: V Chandran, T Watts, A-C Gingras, N Haroon, R Inman, A McGeer, M Silverberg, B Kuriya McMaster: D Bowdish, M Larche, S. Collins, J Marshall, S Garner, I Nazy Manitoba: C Hitchon Waterloo: R Cook Calgary: G Kaplan, C Barnabe, B Hazelwood Memorial: P Rahman Sherbrook: G Boire Laval: P Fortin	component $630,120.00 +18% OH)
2021-2022	Timing of second dose of SARS-CoV2- mRNA vaccine on the Immunologic and fuNctional Antibody Responses Generated in Healthcare workers (TIMING Study). PHRI Internal Funding competition. Competitive. Co-PI: D Leong Co-Applicant: D Bowdish, I Nazy, M Miller, M Loeb, Z Chagla, D Mertz, P Kim, J Denburg	$79,000
2021-04-01- 2026-03-30	Macrophage senescence impairs phagocytosis and phagosome function National Science & Engineering Research Council of Canada (NSERC) PI: D Bowdish	$250,000 ($50,000/year)
2020-03-31- 2023-02-27	Understanding whether the microbiome and upper respiratory tract immune responses contribute to infection or protection from infection, with SARS-CoV2 W. Garfield Weston Foundation PIs: D Bowdish, M Surette	$298,940
2020-03-31- 2023-02-27	Harnessing the microbiota to promote healthy aging and prevent respiratory infections in older adults W. Garfield Weston Foundation PIs: D Bowdish, M Surette	$1,000,000
2020-01-01- 2025-01-01	Preclinical Studies in Aging Laboratory Canadian Foundation for Innovation John Evans Leadership Fund and Ontario Research Fund-Research Infrastructure (ORF-RI) Ministry of Economic Development, Job Creation and Trade (MEDJCT)	$356,483 (from CFI) $356, 483 (from ORF-RI
2018-09-01- 2023-08-31	Age-associated inflammation alters myeloid cell development and function Canadian Institutes of Health Research Co-Investigators: M Rauh, D Winter	$983,025
2015-2023	Solving the antibiotic resistance crisis Ontario Research Fund Principal Applicant: Gerry Wright	$10,655,996

	Co-applicants: D Bowdish, E Brown, L Burrows, B Coombes, A Capretta, N Magarvey, M Surette, D Mertz, A Savchenko, M Organ	$75,000/yr to Bowdish in years 1-3
Previously held:		
2020-12-15-2023-12	Immunologic and facility-wide predictors of COVID-19 risk in long term care Canadian Immunity Task Force Co-PIs: A Costa, D Bowdish	$5,000,000
2020-05-31-2021-04-31	Airway and systemic cellular and immune responses and lung mechanics in Covid-related acute lung injury Hamilton Academic Health Sciences Organization Co-Principle Applicants: M Mukherjee, P Nair Co-applicants: D Bowdish, M Smieja, T Ho	$89,405
2020-05-25-2021-04-31	Immune phenotyping of symptomatic and asymptomatic SARS-CoV-2 infection in pregnant women admitted for delivery in Hamilton Ontario Hamilton Academic Health Sciences Organization PIs: J Denburg, D Bowdish	33,000
2015-05-01-2020-04-31	Interplay between inflammation & impaired anti-bacterial immunity in the elderly Ontario Early Researcher Award	$60,697.27
017-08-01-2019-08-31	Mining the microbiota for novel immunotherapies Boris Family Foundation Collaborators: M Surette, M Loeb	$100,000
2017-06-01-2019-06-01	Dysregulation of regulatory T cells during chronic infection in muscle R21 NIH #RAI128284A PI: E Wohlfert Collaborator: D Bowdish	$330,000
2017-09-01-2018-09-01	Novel probiotics to promote healthy aging and reduce susceptibility to respiratory infections in the elderly Weston Family Microbiome Initiative Garfield Weston Family Foundation Co-PI: M Surette	$0
2016-04-01-2018-03-31	Province of Ontario Neurodevelopmental Disorders Network (POND) Ontario Brain Institute - Interdisciplinary Program PI: S Georgiades Co-PI: J Foster Collaborator: D Bowdish	$150,000 $50,000 to Bowdish

2017-06-01- 2018-05-31	Comparing the regulatory networks underlying inflammation in arthritis & aging Arthritis National Research Foundation (ANRF) PI: D Winter Co-Investigators: D Bowdish, H Perlman	$27,500
2015-07-01- 2016-06-30	Host-pathogen strain specificity as determinant in complicated pneumonia and pleural empyema Ontario Lung Association – Grant in Aid PI: M Surette Collaborator: D Bowdish	$100,000 ($5,000 to Bowdish)
2016-08-01- 2017-07-30	The aging microbiome as a risk factor for developing pneumococcal pneumonia in mid- to late-life Ontario Lung Association Principal Applicant: D Bowdish	$49,500
2012-09-01- 2018-03-01	Macrophage function changes with age and contributes to susceptibility to infectious disease Canadian Institute for Health Research (CIHR) Principal Applicant: D Bowdish	$48,023
2012-09-01- 2017-08-01	The viral toxin: understanding binding, internalization and signaling of dsRNA Canadian Institute for Health Research (CIHR) Co-applicants: D Bowdish, K Mossman	$1,047,395
2014-06-01- 2017-05-31	Enhanced scientific capacity for risk assessment of the pathogenicity potential of microbes associated with biotechnology Health Canada - Health Canada Biotechnology Initiative Principal applicants: PS Shwed, AF Tayabali Collaborator: D Bowdish	$ 709,615 $1,500 to Bowdish
2014-01-01- 2015-01-01	Establishing age-related chronic inflammation as a modifiable risk factor for poor immune function in the elderly Labarge Optimal Aging Initiative Co-applicants: D Bodwish, M Loeb, C Verschoor, G Pare	$316,000/yr x 3yrs $6,000 to Bowdish
2013-01-01- 2016-01-01	Probiotics to Prevent Respiratory Infections in the Elderly Labarge Optimal Aging Initiative Co-applicants: D Bowdish, M Loeb, J Bramson, M Surette, J Johnstone, P Nair	$58,000
2010-09-01- 2015-08-31	Respiratory tract microbiome dynamics and the interplay of commensal bacteria with resident pathogens Canadian Institutes for Health Research (CIHR) – Emerging Team Grant Co-applicant: D Bowdish, M Surette	$200,000

2010-09-01- 2015-08-31	Drug Discovery in a post-antibiotic world: Infrastructure for development of novel immunomodulators Canadian Foundation for Innovation (CFI) - Leaders Opportunity Fund Principal applicant: D Bowdish	$500,000
2010-05-01- 2015-04-31	Novel signaling motifs in macrophage pattern recognition receptors National Science & Engineering Research Council of Canada (NSERC) Principal applicant: D Bowdish	$352,693
2014-10- 2015-05	SSI treatment as a novel immunotherapeutic Industrial Contract – Qu Biologics	$33,000/yr x 5yrs
2013-03-01- 2015-02-28	Probiotics: Prevention of Severe Pneumonia and Endotracheal Colonization Trial (PROSPECT): A feasibility and proof of concept pilot trial Canadian Institute for Health Research (CIHR) PI: D Cook Co-applicants: D Bowdish, D Heyland, J Johnstone, J Marshall, M Meade, M Surette, L Thabane	$63,734.40
2012-03-01- 2015-02-28	Role of MARCO in Susceptibility & Resistance to Tuberculosis Subcontract Lead PI: K Sakamoto (University of Georgia)	$146,863/yr
2014-03-31- 2014-09-01	Lung Cancer Diagnostic (supporting Kyle Novakowski (MSc student) and Dr. Chris Verschoor (PDF)) Connect Canada Internship Awards – Miami Mice Co-applicant: D Bowdish	$29,000/yr x 3 yrs
2014-03-31- 2014-09-01	Quantitative immunoassay for TLE1 protein National Science & Engineering Research Council of Canada (NSERC) - Engage, Industrial collaboration with Miami Mice Principal applicant: D Bowdish	$20,000
2013-05- 2013-12	SSI treatment as a novel immunotherapeutic Industrial Contract – Qu Biologics	$25,000
2012-08-01- 2013-08-01	Age-associated pneumonia results from impaired immune control of nasopharyngeal carriage Ontario Lung Association-Pfizer Canada Research Award Principal applicant: D Bowdish	$25,200
2012-01-01- 2012-05-01	Effects of pneumococcal colonization on SSI therapy Industrial contract- Qu Biologics (Vancouver).	$50,000
2010-07-01- 2013-06-01	Control of *Streptococcus pneumoniae* carriage National Institutes of Health (NIH) – RO1 subcontract Co-applicant: J Weiser	$25,575.68 x 0.5yrs

2011-01-01– 2011-12-31	Intranasal administration of Linezolid to prevent seasonal & post-influenza pneumococcal pneumonia in the elderly ASPIRE-Pfizer - New Investigator Award	$50,000/yr x 3yr
2010-06-01- 2011-05-31	Molecular mechanisms of monocyte/macrophage immunosenescence and susceptibility to *Streptococcus pneumoniae* infection in the nursing home elderly Canadian Institutes for Health Research (CIHR) – Catalyst Grant Principal applicant: D Bowdish	$100,000/1yr
2010-12-01- 2011-12-01	Macrophage scavenger receptors – novel recognition receptors for *Mycobacterium tuberculosis* Ontario Lung Association - Grant-in-Aid Principal applicant: D Bowdish	$50,000/1yr
2010-03-01- 2011-03-31	The role of macrophage function in prevention of *Streptococcus pneumoniae* infection in the nursing home elderly CIHR (Canadian Institutes of Health Research) Principal applicant: D Bowdish	$50,000/1yr
2010-05-01- 2011-04-31	Clinical markers of monocyte immunosenscence Institute for Infectious Disease Research (IIDR) Co- applicants: D Bowdish, J Bramson, J Gauldie	$100,000/yr x 1 yr
2009-05- 2010-06	Colonization vs Infection: Why aren't macrophage responses to *Streptococcus pneumoniae* immunizing? Institute for Infectious Disease Research, McMaster University Principal applicant: D Bowdish	$10,000/yr x 1 yr

Patents

- Biological Membrane-Based Sensor. MC Rheinstädter, S Himbert, RJ Alsop, JM Moran-Mirabal, K Saem, DME Bowdish. U.S. Provisional Pat. Ser. No. 62/413,652, filed October 27, 2016
- Structured Glassy Surfaces for Use as Substrates for Immune Cell Assays. DME Bowdish, J Moran-Mirabal, J Boyle, Y Zhu. Disclosure agreement filed May 2016.
- Blockade of TNFalpha for treatment of age-related tissue damage and impaired anti-bacterial activity. D Bowdish, A Puchta. Provisional patent filed May 23rd, 2014, Serial No. 62/002,40, 6.
- Effectors of innate immunity. REW Hancock, B Finlay, M Scott, D Bowdish, C Rosenberger and JP Powers. U.S. provisional patent No 60/336,632, filed Dec. 3, 2001 Full US application 10/308,905 filed Dec. 2, 2002; published Sept 16, 2004. US patent 7,507,787, granted March 24, 2009.
- Effectors of innate immunity. REW Hancock, B Finlay, M Scott, D Bowdish, C Rosenberger , JP Powers, N Mookherjee, J Yu. CIP of US application 10/308,905; Application no. US11/400,411, filed April 7, 2006.
- Effectors of innate immunity. REW Hancock, B Finlay, M Scott, D Bowdish, C Rosenberger, JP Powers. South African Patent No. 2004/4919, granted May 31, 2006.
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11 Prabhudas M, **Bowdish D**, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J, Means TK, Moestrup SK, Post S, Sawamura T, Silverstein S, Wang XY, El Khoury J. *Standardizing scavenger receptor nomenclature*. J Immunol. 2014 Mar 1;192(5):1997-2006. doi: 10.4049/jimmunol.1490003. PMID: 24563502

12 Whelan FJ*, Yap N, Golding B, **Bowdish DME.** *A guide to bioinformatics for immunologists*. Frontiers in Immunology. 2013. 4, 416. doi: 10.3389/fimmu.2013.00416. PMID: 24363654 *****2nd most highly downloaded paper from this journal in 2014

13 Dorrington MG*, **Bowdish DME**. *Immunosenescence and novel vaccination strategies for the elderly*. Frontiers in Immunology (Editor Dr. Lorne Babiuk). 2013. doi:10.3388/immu.2013.00171. PMID: 23825474

14 **Bowdish DME,** Gordon S. *Conserved domains of the class evolution and function*. Immunol Rev. 2009 Jan;227(1):19-31. PMID: 19120472

15 **Bowdish DM**, Loffredo MS, Mukhopadhyay S, Mantovani A, Gordon S. *Macrophage receptors implicated I the "adaptive" form of innate immunity*. Microbes infect. 2007 Nov-Dec; 9(14-15): 1680-7. PMID: 18023392

16 Gibbons MA, **Bowdish DME**, Davidson DJ, Salleneuve JM, Simpson AJ. *Endogenous pulmonary antibiotics*. Scott Med J. 2006 May;51(2):37-42. PMID: 16722137

17 **Bowdish DME,** Hancock REW. *Anti-endotoxin properties of cationic host defence peptides and proteins*. J. Endotoxin Res. 2005. 11(4):230-6. PMID: 16176660

18 **Bowdish DME,** Davidson DJ, Hancock REW. *A re-evaluation of the role of host defence peptides in mammalian immunity*. Curr Protein Pept Sci. 2005 Feb;6(1):35-51. PMID: 15638767

White Papers/Policy reports/Guideline:

1. Canadian Thoracic Society- Information and Guidance for Respiratory Health Care Professionals on COVID 19 Vaccination. https://cts-sct.ca/wp-content/uploads/2021/05/Ver-5.0_CTS-COVID-19-Vax-Guide_May-25_2021.pdf
2. Data used for the development of the National Advisory Council on Immunization's guidelines for vaccination of vulnerable populations:
    a. NACI rapid response: Booster dose in long-term care residents and seniors living in other congregate settings [2021-09-28] https://www.canada.ca/en/public-health/services/immunization/national-advisory-committee-on-immunization-naci/statement-september-28-2021-booster-dose-long-term-care-residents-seniors-living-other-congregate-settings.html
    b. Recommendations on the use of COVID-19 vaccines [2021-10-22] https://www.canada.ca/en/public-health/services/immunization/national-advisory-committee-on-immunization-naci/recommendations-use-covid-19-vaccines.html
3. National Institute on Ageing. (2019). As One of Canada's Top Killers, Why Isn't Pneumonia Taken More Seriously? Toronto, ON: National Institute on Ageing White Paper.https://www.ryerson.ca/nia/white-papers/why-isnt-pneumonia-taken-more-seriously.pdf
4. The Lung Association Ontario in partnership with the National Institute on Ageing. (2019) "Prevention: The Best Medicine. The Importance of Immunization to the Health of Seniors."
5. The Lung Association Ontario. (2019) "Paving the way for Immunization Innovation".

Publications for a lay/broad audience

1. **Teskey G, Bundalovic-Torma C, Bowdish DME**. *Age-associated inflammation alters the aging trajectory*. Biochem (Lond) 1 February 2019; 41 (1): 22–25. doi: https://doi.org/10.1042/BIO04101022

Figures

1. **S Makaremi, H Luu, JP Boyle**, Y Zhu, C Cerson, **DME Bowdish**, JM Moran-Mirabal… - Advanced Materials Interfaces, 2019. Plasma Membrane: The Topography of Silica Films Modulates Primary Macrophage Morphology and Function (Adv. Mater. Interfaces 21/2019)
2. S. Gordon. "Leukocyte migration" and "Phagocytosis & Autophagy" for: Macrophages and Phagocytosis. In: Fundamental Immunology 6th Ed. 2007. W. Paul Lippincott Raven. Publ. Philadelphia. Chapter 17.

Presentations (Invited):

1. Hema-Net Serosurveillance Meeting. "Assessing population representativeness of serosurveillance data: Lessons learned from the COVID-19 in LTC study"McGill University, Montreal, Quebec. February 14-16th.
2. Better Breathing Week. Toronto, Ontario "Vaccines for people living with lung disease." January 17th. 2024.
3. Division of Respirology Research Rounds. Firestone Institute for Respiratory Health. McMaster University. "The Firestone Institute for Respiratory Research – A year in review". January 9th, 2024.
4. University of Ottawa Heart Institute Weekly Research Conference. Ottawa, Canada. "Infections and vaccinations in older adults". November 27, 2023[1]
5. Future of Vaccinology Symposium. McMaster University, Canada. "COVID-19 vaccinations in vulnerable older adults." October, 13, 2023.
6. Occupational Health Clinics for Ontario Workers. Ontario, Canada. "COVID-19 infections and vaccinations in older adults: Managing infections and reinfections". October 6, 2023.
7. Hamilton Third Age Learning. Hamilton, ON. "The perils of being born in the Fall.". September 26, 2023.
8. Oxford Macrophage Symposium. "Macrophages, infection and immunity in youth and age." Oxford, U.K. September 13,2023.
9. COVID-19 Immunology Consortium-BC Research Day. Vancouver, Canada. "Lessons learned from studying COVID-19 vaccinations and infections in long-term care". June 16, 2023.
10. McMaster Internal Medicine Research Day. Keynote presentation. McMaster University, Canada. "Finding your scientific voice and using it for the greater good". May 17, 2023.
11. COVID-19 Immunity Task Force. Plenary presentation on Infections in Vulnerable Populations. Co-presenters Drs. Sonia Anand, Upton Allen. Vancouver, Canada. March 8, 2023
12. Firestone Institute for Respiratory Health. "COVID-19 infections & vaccinations in vulnerable populations". February 23, 2023
13. Banff Inflammation Workshop. Banff, Alberta. "Early life adversity alters immunity". January 26, 2023.
14. Canadian Association for Laboratory Animal Science Webinar. Virtual. "Understanding non-genetic factors associated with healthy and unhealthy aging using the PreClinical Studies in Aging Lab". January 19, 2023
15. Infection and Immunity Research Forum. Western University. Keynote Speaker. "The myelopoiesis of Ms Macrophage – tales of a career in infection and immunity." November 4, 2022.
16. Centre for Vaccine Preventable Diseases Symposium – Vaccination in Older Adults. University of Toronto. "COVID-19 Vaccination in Long-term care". September 16, 2022.
17. 12th International Symposium on Pneumonia and Pneumococcal Disease (ISPPD). "Age-associated Inflammation Potentiates Pneumococcal Disease". June 6, 2022
18. University of Toronto Senior College Symposium – What have we learned from the pandemic. "COVID-19 vaccinations in vulnerable populations. Tales form a scientist turned science communicator." April 20, 2022
    i. Presentation available: https://www.youtube.com/watch?v=FUhABJCajKQ&list=PLqkMLowL3YG4gb-LR3wHMpsdb132dCBXM&index=4
19. Institute for Research on Public Policy. "Health tech: The politics and policies of remote rehabilitation". April 1st, 2022. Panelist. https://irpp.org/irpp-event/health-tech-the-politics-and-policies-of-remote-rehabilitation/
20. Thrombosis & Atherosclerosis Research Institute (TAARI). Cardiovascular Research Seminar Series. "The aging immune system contributes to lung infections". March 2, 2022.
21. Better Breathing Week. COVID-19 in Long Term Care: Where Policy, Research and Practice Meet & COVID-19 Vaccine Dosing Intervals: Immune response "COVID-19 infections and vaccinations in long-term care and retirement homes". January 28, 2022

22. Hamilton Health Sciences. “Building confidence in vaccines” with Dr. Cora Costanescu (Alberta Health Services) and moderated by Dr. J. Pernica. January 26, 2022
23. Ontario Advisory Committee on Immunization. “COVID-19 infections & vaccinations in long-term care homes”. December 13, 2021
24. Jackson Laboratories invited speaker series. “The microbiome and unhealthy aging – cause or consequence?”. Novembers 30, 2021
25. Université Laval- Quebec Heart and Lung Institute Seminar Series. “The aging immune system contributes to lung infections”. November 18, 2021
26. COVID-19 Immunity Task Force/Public Health Agency of Canada. “COVID-19 vaccinations & infections in long-term care”. October 28, 2021
27. Masoro-Barshop Conference on Aging. Virtual Conference – The Aging Immune System. “Systemic inflammation inhibits macrophage function and predisposes to infections”. October 15, 2021.
28. Systems Immunology in Aging and Complex disease. The Jackson Laboratories. Virtual Conference. “The microbiome in unhealthy aging”. September 9, 2021.
29. American Association of Immunologists. Summer School in Immunology. Washington, USA. “Myeloid cells in health & disease”. July 27, 2021.(Virtual seminar, due to COVID-19 restrictions).
30. AstraZeneca Mexico. Invited lecture for clinicians and respirologists. “Innate immunology of the lung”. July 2021
31. Vanderbilt University Seminar Series. “Age-related changes in immunity & the microbiome predispose to infections” April 18, 2021.
32. iWISE (Women in Science and Engineering) conference. “The myelopoeisis of @MsMacrophage: My career in microbes, macrophages, and more recently COVID-19”. Keynote. March 25, 2021
33. University of Birmingham. Institute of Inflammation and Ageing. “Age-related changes in immunity & the microbiome pre-dispose to infections”. March 10, 2021
34. Johns Hopkins Bloomberg School of Public Health. Department of Molecular Microbiology and Immunology “Age-related changes in immunity & the microbiome pre-dispose to infections”. February 11, 2021.
35. Wake Forrest University. Microbiology & Immunology Department Seminar Series “Age-associated inflammation impairs macrophage anti-pneumococcal immunity”. November 19, 2020
36. USC Leonard Davis School of Gerontology Seminar series. “Age-related microbial dysbiosis alters innate immune function and increases risk of infection”. October 21, 2020. (Virtual seminar due to COVID-19 restrictions).
37. University of California San Francisco Aging Science Symposium: Mechanisms of declining protective immunity despite heightened chronic inflammation during aging. “Secrets of the super-centenarians: the immunology of longevity”. October 15, 2020. (Virtual seminar due to COVID-19 restrictions).
38. Canadian Institute for Advanced Research (CIFAR). “Microbial dysbiosis and unhealthy aging: Cause or consequence?”. September 30, 2020. (Virtual seminar due to COVID-19 restrictions).
39. Yale Pulmonary Research Conference. Yale University. “Secrets of the super-centenarians: The immunology of aging well”. September 24, 2020. (Virtual seminar due to COVID-19 restrictions).
40. VII International Meeting of Biosciences and Physiopathology. “The unusual immunology of COVID-19/SARS-CoV-2”. September 16, 2020. (Virtual seminar)
41. 24th Annual Immunet Research Day. University of Alberta. Keynote Presentation. “The myelopoesis of @MsMacrophage: My career in macrophages and microbes”. August 21, 2020. (Virtual seminar due to COVID-19 restrictions).
42. The Aging in Critical Care Interest Group & Lung Aging Research Working Group. “Promoting recovery in critically ill older adults with COVID-19: Bench to bedside”. July 30, 2020. Panelist with Lekshmi Santhosh, MD and Leah Witt, MD.(Virtual seminar, due to COVID-19 restrictions). https://www.thoracic.org/professionals/clinical-resources/critical-care/journal-club/promoting-recovery-in-critically-ill-older-adults-with-covid-19bench-to-bedside.php

43. American Association of Immunologists. Summer School in Immunology. Washington, USA. “Myeloid cells in health & disease”. July 27, 2020.(Virtual seminar, due to COVID-19 restrictions).
44. Life Sciences Seminar series. University of Nottingham. Nottingham, UK. “The role of inflamm-aging and immunosenescence in aging immune responses”. July 17,2020 (Virtual seminar, due to COVID-19 restrictions).
45. Lung Health Foundation. Toronto, ON. “Everything you wanted to know about SARS-CoV-2/COVID-19 but were afraid to ask”. June 25, 2020. (Virtual seminar, due to COVID-19 restrictions).
46. Infectious Disease-IIDR rounds. McMaster University. “The unusual immunology of SARS-CoV-2 infection”. June 2, 2020. (Virtual seminar due to COVID-19 restrictions).
47. Infection and Immunity Seminar series. University of Calgary. Calgary, Alberta. “Age-related microbial dysbiosis alters innate immune function and increases risk of infection”. March 5, 2020.
48. Biology of Aging Seminar Series. Yale University. New Haven, Connecticut. U.S. “Age-related microbial dysbiosis alters innate immune function and increases risk of infection”. February 3, 2020.
49. Meakins-Christie Laboratories Seminar Series. McGill University, Montreal, Canada. “Age-related changes in the microbiome and susceptibility to pneumonia”. January 28, 2020.
50. British Thoracic Society Annual Winter Meeting. London, U.K. “Inflammageing and the microbiome in the lung”. December 4, 2019.
51. Department of Biomedical Sciences. Tufts University. Boston, US. “Microbial dysbiosis increases age-associated inflammation and susceptibility to pneumococcal infections”. November 12, 2019.
52. Centre for Critical Illness Research. Lawson Health Research Institute. University of Western Ontario. London, Ontario. “Age-associated inflammation contributes to susceptibility to infection”. October 29, 2019.
53. Biology of Ageing Symposium. Institute for Molecular Bioscience, University of Queensland. Brisbane, Australia. "Microbial dysbiosis drives age-associated inflammation and macrophage dysfunction". September 20, 2019.
54. 14th World Congress on Inflammation. Sydney, Australia. “The aging microbiome contributes to age-associated inflammation and immunosenescence”. September 16, 2019.
55. Gordon Research Conference on Phagocytes. Waterville Valley, NH. USA. “Macrophage phagocytosis changes with age and chronic inflammation”. June 6, 2019.
56. Washington University in St. Louis, Immunology Seminar Series. St. Louis, MO. “Age associated inflammation and microbial dysbiosis impair macrophage function”. May 24, 2019.
57. International Infection, Immunity and Inflammation Conference (I4C). University of British Columbia. Vancouver, B.C. Canada. “Age-associated inflammation, macrophage function and longevity”. May 14-15, 2019.
58. Oklahoma Geroscience Symposium -The Role of Inflammation in Aging and Age-Associated Diseases, Oklahoma City, OK. “Role of the microbiome in inflammaging”. April 24, 2019.
59. University Health Network. Toronto, ON. “Age-associated inflammation and microbial dysbiosis alter the aging trajectory”. April 15, 2019.
60. Ottawa Centre for Infection, Immunity and Inflammation (CI3). Ottawa, ON. “The aging microbiome contributes to age-associated inflammation and immunosenescence”. March 29, 2019.
61. University of Toronto. Department of Immunology. Toronto, ON. “Age-associated inflammation and the microbiota alter monocyte and macrophage function”. February 21, 2019.
62. McMaster’s Central Animal Facility. “The aging immune system”. February 14, 2019.
63. Farncombe Insitute. McMaster University. “Microbial dysbiosis and age-associated inflammation”. February, 12, 2019.
64. North American Microbiome Conference. Washington, D.C. “Employing aged mice as a model to study the long-term relationship between the immune system and the microbiome”. February 6th, 2019.
65. Better Breathing Conference. Toronto, Ontario. “Pneumonia in older adults”. January 26, 2019.

66. Chang Gung Memorial Hospital. Taipei, Taiwan. “Age-related microbial dysbiosis contributes to systemic inflammation and impaired macrophage function”. January 16, 2019.
67. McMaster Allergy & Immunology Rounds. “Myeloid immunophenotyping as a measurement of inflammatory tone”. December 14, 2018.
68. Cedars-Sinai Immunology Seminar Series. Los Angeles, California. “Microbial dysbiosis drives age-associated inflammation and myeloid dysfunction”. November 29, 2018.
69. Roswell Park Comprehensive Cancer Centre. Department of Immunology. Buffalo New York. “Microbial dysbiosis drives age-associated inflammation and myeloid dysfunction”. November 20, 2018.
70. Society of Leukocyte Biology/ International Endotoxin Society Joint Meeting. Myeloid cells: Development, environment and inflammation. Chandler, Arizona, USA. “Microbial dysbiosis drives age-associated inflammation and impairs myeloid development”. October 14-16, 2018.
71. University of Alabama at Birmingham Microbiology and Immunology Seminar Series. “Age, inflammation and microbial dysbiosis contribute to susceptibility to pneumonia”. July 10th, 2018.
72. 31st Annual Canadian Society of Immunology Conference. London, ON. “Age-associated Inflammation Impairs Monocyte and Macrophage Development and Function”. June 2, 2018.
73. Banff Conference on Infectious Disease. Banff, Canada. “Age-associated inflammation increases susceptibility to *Streptococcus pneumoniae* infection”. May 25, 2018.
74. 2018 Canada Gairdner Global Health Lecture Series -Planetary health through food and microbes. “Novel strategies for preventing infectious disease”. Friday May 4, 2018.
75. Canadian Trials Network Annual Meeting. Keynote talk “Aging less than gracefully: The microbiome drives age-associated inflammation and immune dysfunction.” April 24, 2018.
76. University of Manchester. Department of Immunology Seminar Series. “Age-associated inflammation and microbial dysbiosis impair macrophage function and anti-microbial immunity”. February, 27, 2018.
77. Experimental Human Pneumococcal Colonization (EHPC) Mid Term Programme Meeting. Liverpool, U.K. “Age-associated inflammation alters host defence to *Streptococcus pneumoniae*.” February 28, 2018.
78. Biology Department, University of Waterloo. “Pneumococcal infections: Lessons from the four corners of the world”. February 9, 2018.
79. Environmental Drivers of Aging: Proteostasis, Metabolism and Signalling Conference. NorthWestern University. “The aging microenvironment dictates monocyte phenotype and function”. January 12, 2018.
80. MRC Institute for Inflammation Research. Edinburgh, U.K. “Age-associated inflammation & microbial dysbiosis impair macrophage function and anti-microbial immunity”. November 3, 2017.
81. International Conference on Respiratory Pathogens – The Molecular Biology of Bacterial and Viral Respiratory Pathogens. University of Greifswald, Rostock Germany. Keynote speaker. “Age and inflammation predispose to *Streptococcus pneumoniae* infection”. November 2, 2017.
82. Institute Pasteur. Immunology Seminar Series. “Metchnikoff’s theory of aging: Intestinal permeability drives age-associated inflammation and susceptibility to infection”. October 13, 2017.
83. Sick Kids Hospital. Molecular Medicine Seminar Series. Toronto, ON. “The microbiome drives age-associated inflammation and susceptibility to pneumonia”. October 2, 2017.
84. Research Rounds. Firestone Institute of Respiratory Health. “Monocyte maturation is altered by age, inflammation and the microbiome and contributes to susceptibility to pneumonia”. September 19, 2017.
85. Stanford University Immunology and Transplantation Department Seminar Series. “The microbiome drives age-associated inflammation and increases susceptibility to pneumonia”. August 16, 2017.
86. Invited Seminar. Abbvie Immuno-oncology group. Redwood City, California. “Age-associated inflammation alters monocyte development: implications for immunotherapy”. August 15, 2017.
87. Infectious Diseases/Microbiology Research Day. Keynote presentation. University of Toronto. “The microbiota drives age-associated inflammation and susceptibility to pneumonia”. June 20, 2017.
88. MedImmune, Gaithersburg, MD. “Age-related inflammation: causes and immunological consequences”. Mary 23, 2017.
89. American Thoracic Society Annual meeting. “Lung infection in an aging population”. May 22, 2017.

90. Aging & Immunity Satellite Meeting to the American Association of Immunology. Bethesda, U.S. "Infections and changes in the microbiome with aging". May 10, 2017
91. Quebec Annual Scientific Meeting for the Genome Quebec/ le Ministere e l'Economie de la Science et de l'Innovation (MESI) Scientific Advisory Board Meeting for project "Exploiting plant-made vaccines to protect the elderly against respiratory infections" Keynote presentation. "Age-associated inflammation alters inflammatory responses to respiratory pathogens". May 4, 2017.
92. International Research Symposium Centred on Understanding and Preventing Infection in Children. Vancouver, BC. "The perils of inter-generational transfer of *Streptococcus pneumoniae".* April 28, 2017.
93. NorthWestern University. Division of Pulmonary and Critical Care Medicine Invited Speaker Series. "The microbiome drives age-associated inflammation and myeloid immunosenescence". April 3, 2017.
94. Queen's University. Department of Pathology & Molecular Medicine Seminar Series. "Aging less than Gracefully: Age-associated inflammation alters monocyte development and increases susceptibility to infection". February 22, 2017.
95. Wilfred Laurier University. Department of Biology Seminar Series. "Aging less than gracefully: Age-associated inflammation alters monocyte development and increases susceptibility to infection". January 20, 2017.
96. Firestone Respirology Research Christmas Rounds. "Kiss your grandmother: The perils of intergenerational contact during the holiday season". December 13, 2016.
97. University of Toronto Immunology Speaker Series. "Age, inflammation and microbial dysbiosis conspire to impair anti-microbial immunity". December 5, 2016.
98. Society for the Immunobiology of Cancer Annual International Meeting. "Myeloid-derived suppressor cells (MDSC), age and cancer". November 13,2016.
99. University of Maryland (UMB) Invited Speaker Series. "Aging less than gracefully: Inflammation and the microbiome impair antimicrobial activity". November 9, 2016.
100. University of Georgia (UGA) Center for Vaccines & Immunology Seminar Series. Georgia, Alabama. "Age, infection & inflammation: More than the sum of their parts". October 17, 2016.
101. The Society for Leukocyte Biology's 49th Annual Meeting. "The aging microbiota reprograms inflammatory Monocytes". September 15-17, 2016.
102. Louisiana State University Invited Speaker Series. Baton Rouge, Louisianna. "Metchnikoff's theory of aging: Intestinal permeability drives age-associated inflammation and susceptibility to infection". September 8, 2016.
103. Canadian Critical Care Trials Network. Halifax, NS. "Mechanistic sub-study: Circulating inflammatory and immunomodulators in critically ill patients in the PROSPECT trial". June 14, 2016.
104. PROSPECT Launch and Investigator meeting. Informational sessions for research co-ordinators and nurses in the PROSPECT trial. Halifax, NS. "The potential of probiotics" and "Immunology for the uninitiated". June 13, 2016.
105. McMaster Geriatric Grand Rounds. Juravinski Hospital, Hamilton, ON. "Age–associated inflammation & susceptibility to *Streptococcus pneumoniae* infection". April 11, 2016.
106. Gordon Conference on Acute Respiratory Infections. Galveston, Texas. "Novel vaccination strategies for the elderly". February 26, 2016.
107. Invited Speaker series. Department of Microbiology & Immunology. University of Buffalo. "Age, infection & inflammation: More than the sum of their parts". November 18,2015.
108. Invited speaker series. Department of Biology, Ryerson University. "Aging less then gracefully: How macrophage function declines with age and contributes to susceptibility to infection". October 22, 2015.
109. McMaster Institute of Geroscience Inaugural Symposium. "Age, infection & inflammation: More than the sum of their parts". October 1, 2015.
110. Allergy & Immunology Rounds. McMaster University. "The microbiome drives age-associated inflammation". May 22, 2015

111. Province of Ontario Neurodevelopmental disorders network Science Day. “Inflammation, infection, cognition and age”. May 8, 2015
112. Ottawa-Carleton Institute of Biology Student Conference. Keynote speaker. “Aging gracefully: How aging and immunity intersect”. April 29, 2015.
113. Buffalo Immunology Conference. Maryville NY. “The aging microbiome drives age-associated inflammation”. April 23-24, 2015.
114. EMPHASIS seminar series, McMaster University. Keynote speaker. “The metabolism of age-associated inflammation”. April 23, 2015.
115. University of Georgia Invited Speaker Series. “The role of phagocytic receptors in *Streptococcus pneumoniae* infections”. March 28, 2015
116. Keystone Symposia: Dendritic Cells and Macrophages Reunite. Workshop presentation. Montreal, QC. “Metchnikoff’s theory of aging: Intestinal permeability drives age-associated inflammation and macrophage dysfunction”. Mar 8 -10, 2015.
117. Farncombe Institute of Digestive Health Seminar Series. “Age, inflammation & the microbiota: Why the choices that you make in youth haunt/help you in old age”. January 7, 2015.
118. Biology of Aging Seminar Series. McMaster University, Hamilton, ON. “Aging or inflamm-aging? Which causes immune decline with age?” December 5, 2014.
119. Pfizer sponsored Research Exchange Meeting in conjunction with the Canadian Immunization conference. Ottawa, ON. “Age-associated inflammation contributes to susceptibility to pneumococcal infection”. December 3rd, 2014.
120. Health Canada Invited speaker series. Ottawa, ON. “Aging, inflammation and the microbiome”. December 3rd, 2014.
121. Labarge Optimal Aging Research Day. “Probiotics to prevent respiratory infections in the frail nursing home elderly”. November 10, 2014.
122. Respiratory Research Rounds. Firestone institute. “Age-associated inflammation impairs host-defence towards *Streptococcus pneumoniae*”. November 25 2014.
123. Nathan Shock Aging Center. San Antonio, Texas. “The microbiome drives age-associated inflammation”. October 18, 2014.
124. Albany Medical School. Invited Speaker Series. "Inflammation drives macrophage dysfunction and susceptibility to pneumococcal infection”. Sept 25, 2014
125. University of Guelph. Department of Pathobiology. Invited Speaker Series. “Macrophage scavenger receptors are required for control of colonization in the upper respiratory tract.” Sept 19, 2014
126. British Society of Immunology. Inflammation and Immunity. University of Manchester, UK. “Metchnikoff 's theory of aging: the intestinal microbiota drives age-associated inflammation and impaired macrophage function”. September 9-10, 2014.
127. Federation of Clinical Immunology Societies (FOCIS) Annual Meeting. Chicago, Illinois. “Phagocytosis, Endocytosis and Motility: Scavenger Receptor Functions at the Interface of Homeostasis and Host Defense”. July 25-28, 2014.
128. IIDR Colloquium. McMaster University. “A Breath of Fresh Air: Preventing pneumonia in older adults”. May 30, 2014.
129. The American Association of Immunologist Annual Meeting. Pittsburgh, Pennsylvania. “Scavenger receptors and host-pathogen interactions”. May 2-6, 2014.
130. iSci Symposium Keynote Speaker. McMaster University. “Pneumo a no no: Preventing pneumonia in older adults”. April 3, 2014.
131. Biology of Acute Respiratory Infection Gordon Research Conference. Lucca Italy. Invited speaker. “Pneumonia, inflammation and age”. Feb 23-28, 2014.
132. Lung Association. Better Breathing Conference. Pfizer sponsored keynote lecture “Age, inflammation and pneumonia”. February 2, 2014.

133. McMaster University. Department of Chemistry & Chemical Biology Seminar Series. “Does macrophage metabolism decrease with age?” January 7th, 2014.
134. Vaccine Preventable Disease Program at Hamilton Public Health Services. Hamilton. “Vaccine hesitancy – why has the public turned against vaccines?” November, 21, 2013.
135. University of Western Ontario Department of Microbiology & Immunology Invited Speakers Series. “Macrophage phagocytosis is required for control of nasopharyngeal colonization”. November 7th, 2013
136. McMaster Immunology Research Centre Infection & Immunity Seminar Series. “Macrophages- my favourite cell”. May 29th, 2013.
137. Panel on the Status of Women in Science & Technology. The Canadian Science Policy Centre. Panel participant. April 23rd, 2013. http://www.youtube.com/watch?v=18psL0ilZOk
138. Macrophage Immunobiology, Current Research and Prospects. Kavli Royal Society International Centre U.K. “Macrophage scavenger receptors and immune defence in the upper respiratory tract”. April 13, 2013.
139. MRC Centre for Inflammation Research Seminar Program. Edinburgh, U.K. “Interplay between chronic inflammation, age & anti-bacterial activity”. April 12, 2013.
140. Molecular & Integrative Physiological Sciences seminar series. Harvard School of Public Health. Boston, U.S. “Macrophage phagocytosis - old cells protecting old people”. March 26th, 2013.
141. Respiratory Research Rounds. Firestone institute. “Phagocytosis & age-associated inflammation - an evolutionary approach”. February 12th, 2013.
142. Scavenger Receptors Nomenclature Workshop, Division of Allergy, Immunology, and Transportation, National Institute of Allergy and Infectious Diseases , National Institute of Health, Bethesda, Maryland. "Scavenger recepters and host-pathogen interactions". November 29 - 30, 2012.
143. Demystifying Medicine Seminar Series, McMaster University. "Pneumonia, still the old man's friend?" November 5, 2012.
144. Infection and Immunity Seminar Series, McGill University. "Aging gracefully with the pattern recognition receptors". October 25, 2012.
145. South-Western Ontario Pathogenesis Working Group meeting. University of Guelph. “Those “other” pattern recognition receptors: Phagocytic receptors in host defence to *Streptococcus pneumoniae*”. September 29, 2012.
146. University of Calgary Invited Speaker. “The role of phagocytic receptors in recognition of *Streptoccus pneumoniae*”. September 25, 2012.
147. Firestone Institute. St. Joe’s Research Rounds. “Age-associated changes in monocyte/macrophage function predispose the elderly to infectious disease”. January 27, 2012.
148. Department of Microbiology & Immunology. Queen’s University, Kingston. “Save the grandparents! How an aging immune system predisposes the elderly to pneumonia”. January 19, 2011.
149. Department of Pathobiology. University of Guelph. Invited speaker series. “Mechanisms of immune control of *Streptococcus pneumoniae* in the upper respiratory tract”. January 14, 2011.
150. Society for Leukocyte Biology/International Endotoxin Society Meeting. Vancouver. “Immunosenescent macrophages cannot control *Streptococcus pneumoniae* colonization, resulting in invasive pneumococcal disease". October 7, 2010.
151. Institute of Molecular Biology and Biochemistry (IBMB) Invited Speaker Series. University of Waterloo. “The Scavenger Receptors: Ancient Pattern Recognition Receptors essential for the innate immune response”. January, 26, 2010.
152. Department of Chemical Engineering. Invited Speaker Series. McMaster University. “Macrophage scavenger receptors: roles in adhesion, uptake & motility”. November 11, 2009.
153. IIDR Opening Symposium. McMaster University. “New insights into an ancient disease: A novel macrophage receptor essential for host defence towards tuberculosis”. October 24, 2009.
154. Bench-to-Bedside. McMaster University. “Recurrent pneumococcal infections: A breach in Innate Immunity”. September 21, 2009.

155. Bowdish, D. "Cationic peptides in innate immunity", in Gordon, S. (ed.), Innate Immunity: Host recognition and response in health and disease, The Biomedical & Life Sciences Collection, Henry Stewart Talks Ltd, London. 2009. (online at http://www.hstalks.com/?t=BL0612078-Bowdish)
156. Invited speaker. The University of British Columbia. “The Class A Scavenger receptors are associated with host defence to tuberculosis”. May 15, 2009.
157. Tuberculosis and International Child Health meeting. Imperial College London, UK. “Macrophage receptors in innate immunity: The class A scavenger receptors in mycobacterial and neisserial disease”. March 7, 2008.
158. The Karolinska Institute. Stockholm, Sweden. “Genetic analysis of the scavenger receptor, MARCO”. January 20, 2008.
159. The Whitechapel Society Invited Speaker Series. London, UK. “MARCO – a macrophage receptor associated with mycobacterial infection”. June 14, 2007.
160. Invited Speaker Series. Trinity College, Dublin, Ireland. “MARCO – a macrophage receptor associated with mycobacterial infection”. April 27, 2007.
161. 5th PhD Students Workshop. Riva del Gara, Italy. “Fundamental concepts in immunology”. January 25, 2007.
162. 6th Annual Meeting of the Canadian Society of Microbiology. “LL-37, a host defence peptide with immunomodulatory properties”. June, 21, 2006.
163. Invited Speaker Series. University of Gothenburg, Sweden. “LL-37, a human host defence peptide with immunomodulatory properties”. March 3, 2006.
164. Innate Immunity in Human Disease: BC Research Institute for Children’s & Women’s Health. “LL-37 is a host defence peptide which interacts directly with monocytes & epithelial cells and modulates the innate immune response”. February, 1, 2005.
165. 8th Biennial Conference of the International Endotoxin Society. Kyoto, Japan. “The host defence peptide LL-37 is a multi-functional immunomodulator”. November 15-18, 2004.
166. International Symposium SFB 617 – Molecular Mechanisms of Epithelial Defense. Kiel, Germany. “The involvement of peptides in host defense against infections”. June 11-13, 2004

Community Engagement/Knowledge translation:

Topic: COVID-19

Text Interviews:

i. The Globe and Mail. February 9, 2024. An alarming number of my friends have COVID-19. Are we in another wave? Link
ii. The Hamilton Spectator. February 9, 2024. McMaster creates mouse ICU to study link between dementia and respiratory infection. Link
iii. Toronto Star & syndicates. April 26, 2023. A new COVID subvariant is spreading quickly. Here’s what you need to know. Link
iv. The Record. March 17, 2023. The pandemic taught us that when disaster strikes, we are not equally at risk. Link
v. The Record. March 10, 2023. Deaths fell but hospitalizations surged in Waterloo Region in third year of the COVID-19 pandemic. Link
vi. The Record. January 20, 2023. Hospital cases still high as ‘Kraken’ looms. Link
vii. The Globe and Mail. January 6, 2023. What Canadians need to know about the Omicron ‘Kraken’ subvariant XBB.1.5. Link
viii. The Saxon. December 27, 2022. Here's why public health predicts more COVID-19 infections in 2023. Link

ix. CBC News. December 26, 2022. The virus behind COVID-19 is mutating and immune-evasive. Here's what that means. Link
x. Eminetra. November 10, 2022. Will Doug Ford and Kieran Moore reinstate mask mandates? Link
xi. Guelph Mercury Tribune. October 21, 2022. The sons of Omicron: New COVID variants — what researchers are seeing, and what they worry about. Link
xii. National Post & syndicates. July 30, 2022. Is COVID prematurely aging our immune systems? Link
xiii. Eminetra Canada. July 30, 2022. Is COVID prematurely aging our immune systems? Link
xiv. Toronto Star. April 16, 2022. Everyone has COVID but you? Could you be immune? Link
xv. The Canadian Press. Apr 6, 2022 'A PR disaster': Experts worry messaging may hinder fourth-dose uptake. Link
xvi. The Toronto Star. February 28, 2022. Discussing challenges for people who are immunosuppressed and cannot be fully protected by vaccination. Link
xvii. CBC. February 17, 2022. Discussing challenges for people who are immunosuppressed and cannot be fully protected by vaccination. Link
xviii. CBC. January 28, 2022. Explaining differences in waning immunity between adults and children. Link
xix. CBC. January 13, 2022. Explaining challenges with relying on rapid tests. Link
xx. The Hamilton Spectator. December 30, 2021. Hospitalizations growing as Hamilton reports nearly 1,000 new COVID cases. Link
xxi. The Hamilton Spectator. December 29, 2021. Omicron has reached Hamilton's long-term care homes. What does this mean? Link
xxii. Medical Press. December 16, 2021. COVID-19 vaccine inequity allowed Omicron to emerge. Link
xxiii. The Conversation. December 14, 2021. COVID-19 vaccine inequity allowed Omicron to emerge. (picked up by other news sources) Link
xxiv. CBC Manitoba. November 23, 2021. 4th-wave COVID outbreaks at Manitoba care homes upsetting but 'not surprising,' expert on aging says. Link
xxv. Zoomer. November 8, 2021. Your nutritional blueprint to better health. Link
xxvi. CBC. October 29, 2021. Booster shots, 3rd doses and who needs them. Link
xxvii. Edmonton Journal. August 23, 2021. COVID-19, kids and schools: Why we need to put the risks in perspective. Link
xxviii. Scientific American. Vaccines need not completely stop COVID to curb the pandemic. January 18, 2021 Link
xxix. Global News. October 20, 2020. Lab supply shortages affecting Canadian research on COVID-19. Link
xxx. CBC. September 5, 2020. What the level of COVID-19 immunity in Canada could mean for the vaccine hunt. Link
xxxi. CBC. June 30, 2020. There's no quick post-pandemic fix for Canada's long-term care facilities, say experts. Link
xxxii. Zoomer. May 22, 2020. COVID-19 primer: What we know and what we don't know. Link
xxxiii. CBC. April 21, 2020. Federal government has limited ability to ramp up COVID-19 testing nationwide, say officials. Link
xxxiv. Slate. April 11, 2020. WHO investigating reports of coronavirus patients testing positive again after recovery. Link
xxxv. CTV. April 9, 2020. Lack of resources led to limited COVID-19 testing, but new options are on the way. Link
xxxvi. Energetic City. April 9, 2020. Coronavirus: Why isn't Canada using blood tests for COVID-19? Link
xxxvii. Global News. April 7, 2020. Coronavirus: How B.C. is 'bending' the curve and why there's hope for Ontario and Quebec. Link
xxxviii. Global News. April 2, 2020. Canada may be missing thousands of coronavirus cases, experts say. Link
xxxix. Timmins Today. April 1, 2020. CANADA: How long will coronavirus measures last? Experts say June or July. Link

xl. Global News. April 2, 2020. How families are managing being 2 metres away from grandparents. Link
xli. ABC News. March 30, 2020. 'No metro area will be spared': Officials warn more cities will face outbreaks. Link

Broadcast Interviews:

i. CBC Ontario Today Radio program. November 1, 2023. What do you need to know about the new XBB.1 vaccine?
ii. CBC Ontario Morning Radio Programs. January 20, 2023. Omicron subvariant expected to become dominant.
iii. The Agenda with Steve Paikin. November 14, 2022. What does COVID-19 do to immunity? Link
iv. Ontario Today. September 6, 2022. Syndicated interview answering live questions about COVID-19, immunity and booster shots.
v. CityNews Ottawa. July 15, 2022. Ottawa Public Health reporting increase in COVID-19 hospitalizations. Link
vi. This Matters - The Toronto Star Podcast. COVID restrictions are lifting but some are left behind. March 3, 2022. Link
vii. Sickboy podcast. March 28, 2020. Busting myths about COVID-19. Link
viii. The Future of podcast. April 6, 2020. Coronovirus special. Link
ix. CTV News: Who should get 4th doses? Link
x. Global News. April 16, 2022. How should vulnerable people protect themselves during the 6th wave? Link
xi. The Agenda. March 24, 2022. What to know about the latest variant, BA.2. Link
xii. CBC. April 8, 2022. Is a vaccine that blocks COVID-19 altogether possible? Link
xiii. CBC National. Syndicated series of radio shows for National audiences. March 31, 2022. Who gets 4th doses and why?
xiv. CBC Ontario. Syndicated series of radio shows for Ontario audiences. March 1, 2022. What does the lifting of restrictions mean for the vulnerable?
xv. Instagram Live with Dr. Zain Chagla. August 3, 2021. Vaccine hesitancy. Link
xvi. City News Ottawa. The Rob Snow Show. January 10, 2022. Is deltacron real?
xvii. CBC. January 10, 2022. Series of syndicated interviews on how to best use rapid tests and what it means not to have accurate numbers of COVID infections without PCR tests.
xviii. Global News. March 20, 2022. COVID-19: Should mask mandates continue in Canada? Link
xix. The Conversation. December 19, 2021. Vaccine hesitancy.
xx. Global News: November 29, 2021. How to handle unvaccinated loved ones during the holidays. Link
xxi. CBC. October 4, 2021. Series of syndicated interviews on how influenza and other seasonal viruses intersect with COVID infections.
xxii. Global News: September 27, 2021. Explaining vaccine branding. Link
xxiii. CBC. September 27, 2020. Nurse with long-haul COVID and one vaccine dose asks for exemption from vaccination rules. Link

xxiv. The National (CBC). Explaining who needs a 3rd dose of the COVID vaccine. August 18, 2021
xxv. CBC. August 18, 2021. Series of syndicated interviews on defining a breakthrough infection and who is most likely to have them.
xxvi. CBC. August 3, 2021. Series of syndicated interview on what the 4th wave of COVID will look like in Canada.
xxvii. CBC. July 2021. Series of syndicated interviews on what to expect for cold & flu season.
xxviii. The Agenda with Steve Paikin. April 17, 2020. In search of COVID-19 immunity. Link
xxix. CHCH. April 17, 2020. Researchers at McMaster University looking into different ways to treat the Coronavirus. Link
xxx. Newstalk 1010. October 28, 2020. Explaining why some studies show antibody levels to SARS-CoV-2 decrease with time.
xxxi. CBC. October 5, 2020. Series of syndicated interviews on 'superspreading' COVID-19.
xxxii. CBC. September 24, 2020. Series of syndicated interviews on the COVID response in the 2020 Throne Speech.
xxxiii. Big 96.3. Explaining vaccine strategies: Link
xxxiv. Explaining age-related changes in immune responses Link
xxxv. CBC Radio 1. July 15, 2020. Series of Syndicated interviews on the relative risk of opening bars, nightclubs and restaurants in the context of school re-openings.

Knowledge Translation:

i. 'COVID-19 vaccines webinar' Canadian Arthritis Patient Alliance. With Dr. Inés Colmegna (McGill), Natalie Lalonde (Patient partner) and Dawn Richards (patient partner and president of CAPA). November 4, 2023. https://youtu.be/NmtP34_FVxM?si=zwCV-BUsEQ7CaSb8
ii. "Ageism and the pandemic: How Canada continues to let older adults suffer and die from COVID-19". Article for The Conversation. April 2, 2023.
iii. Study update for research participants in the TIMING study. "The immunology of vaccines & dosing intervals". August 15, 2022.
iv. St Thomas Moore High School. Keynotes speaker for their "Brain Bee" event. "COVID information and misinformation - lessons learned from a scientist turned science communicator". March 18, 2022.
v. "COVID-19 infections and vaccinations in long-term care".
    a. Presentation to COVID-19 Immunity Task Force Field Studies. March 11, 2022.
    b. Presentation to long-term care, retirement and assisted living communities. March 23, 2022
    c. Town hall for research participants and their families. October 20, 2022.
vi. Moderated discussion on how to incorporate vaccination (influenza, pneumococcal, COVID-19) and surveillance into the National Lung Health Agenda. January, 24, 2022.
vii. "Preventing a twindemic through vaccination". (Moderator – speakers include Dr. Mark Loeb, Dr. Jennie Johnstone and Dr. Marek Smieja) Policy forum on the importance of preventing influenza and pneumococcal pneumonia as well as COVID-19 via vaccination. Hosted by the Lung Health Foundation.
viii. "COVID-19 4th doses in long-term care". Partner update for 23 long-term care and retirement communities in our CITF-funded study. December 2021
ix. Q& A session for partners of our CITF-funded grant of vaccine efficacy in First Nations Communities. December 16, 2021
x. "COVID-19 infections & vaccinations in long-term care homes" Town Hall for Schlegel Villages. April 28,2021, September 22, 2021 November 11, 2021

xi. Speaker. COVID-19 Immunity Task Force Seminar. "Protecting Canada's long-term care residents from COVID-19: The evidence behind the policies". October 28, 2021. Link
xii. "COVID-19 infections & vaccinations in long-term care homes" Town Hall for St. Joseph's Villa. June 10, 2021, September 8, 2021
xiii. Explaining infection, immunity and how to keep a healthy immune for the Lung Health Foundation. Link
xiv. Critical Thinking: Vaccines and Hesitancy Workshop presented by CMASTE and GlycoNet (Nov 26, 2021). This workshop covered the topics of vaccines, vaccine hesitancy, and the role that science teachers play as key knowledge translators for students. New resources were developed to help teachers to promote scientific literacy while tacking the issues surrounding the COVID pandemic, especially issues surrounding vaccines. These resources are adaptable to all K-12 levels. Link
xv. Videos with Dr. Bowdish talking about her career as a scientist and tips and tricks she uses for discussions around vaccine hesitancy. Link
xvi. "COVID in long-term care" Province of Ontario Lung Health Caucus meeting hosted by the Lung Health Foundation. November 11, 2020
xvii. "Influenza vaccination in the context of COVID-19/SARS-CoV-2" Province of Ontario Lung Health Caucus meeting hosted by the Lung Health Foundation. September 24, 2020
xviii. "Vaccination & COVID-19". Lung Health Forum for policymakers. July 9, 2020
xix. "Protective immunity wanes in vaccinated long-term care residents" Invited presentation of my CITF funded work to the Vaccine Advisory group, which included representatives from PHAC, PHO and the Ontario Ministry of Health. These data were used to make recommendations for 3rd doses for long-term care residents in Ontario.
xx. "Waning immunity and breakthrough infections and correlates of protection in Long Term Care Residents". Invited presentation of my CITF funded work to Variants of Concern Working Group. Public Health Agency of Canada.
xxi. "Ongoing immunology research in COVID-19". Invited panelist for the Public Health Agency of Canada
xxii. Can schools reopen safely? Infections in kids versus adults. Link
xxiii. Content used by the Globe & Mail: Link
xxiv. Newswise information series for journalists. Link

Topic: Infectious Disease

Text Interviews:

i. Toronto Star. January 22, 2023. Is cold and flu season worse this year? And is 'immunity debt' or 'immunity theft' at play? Link
ii. CBC News. December 16, 2022. Why doctors recommend you get a flu shot this week. Link
iii. The Globe and Mail. November 11, 2022. Return of seasonal flu, RSV and other viruses could spell disaster for older Canadians, experts say. Link
iv. McMaster Daily News. November 15, 2022. McMaster continues to strongly encourage masking & vaccination. Link
v. Toronto Star. November 2, 2022. Next two weeks are critical for getting flu vaccines, Dr. Kieran Moore warns. Link
vi. CBC News. October 20, 2022. McMaster doctors warn upcoming flu season to be more 'intense and pronounced' than previous years. Link
vii. The Canadian Press & syndicates. October 12, 2022. Kids at higher risk of catching flu this season, experts warn. Link

viii. The Toronto Star & syndicates. August 12, 2022. Polio is back on the radar of Canadian health officials. Link
ix. Toronto Star & syndicates. January 24, 2022. Beware of claims that promise to boost your immune system through superfoods or supplements, experts say. Link
x. CBC. December 24, 2021. Omicron's prevalence should shake off COVID-19's lingering stigma, experts say. Link
xi. Saltwire. December 10, 2021. Breakthrough cases rising in P.E.I., but experts say vaccines prevent death, hospitalization. Link
xii. CBC. August 26, 2021. What do we know about breakthrough COVID-19 cases? Experts break down the science. Link
xiii. Maple Ridge-Pitt Meadows News. September 1, 2021. 'Elusive' herd immunity: what will it take to get there with COVID-19? Link
xiv. The Free Press. August 26, 2021. What do we know about breakthrough COVID cases? Experts break down the science. Link
xv. The Toronto Star. (Syndicated in the St. Catharines Standard and elsewhere). November 4, 2019. In the throes of a cold you do what makes you feel better. But are you treating the symptom or the disease? Link
xvi. Chatelaine. January 19, 2016. 8 things you can do every day to avoid the flu. Link
xvii. Everything Zoomer. November 16, 2018. Ask the expert: Canada Research Chair talks flu & pneumonia prevention. Link
xviii. YouAreUNLTD. December 6, 2018. Flu shot? Done! Now get vaccinated against the dangers of pneumonia. Link

Broadcast Interviews:

i. 680 CJOB Radio. December 29, 2022. Risk of the flu.
ii. CBC News. December 16, 2022. Health officials urge flu vaccines before holiday rush. Link
iii. October – November 2018. Spokesperson for the Canadian Lung Association's pneumococcal vaccination campaign. Link
iv. CBC Radio. April 3, 2018. Series of 15 syndicated interviews commenting on a recent study (http://rdcu.be/KwK5) on germs in children's bath toys and our homes in general.

Knowledge Translation:

i. Clinical Roundtable on Vaccines and the Management of COPD. Canadian Thoracic Society. Sponsored by Pfizer Canada (no honorarium or speaking fees received, Pfizer had no control over the content). Moderated a round table discussion on pneumococcal vaccination in patients living with COPD. May 19, 2022.
ii. Dundas Central Public School. Hamilton, ON. "Food Microbiology". March – April, 2019
    - This is a series of lessons designed to complement the grade 5 curriculum and to teach how pH, osmosis, filtration, acidity and pasteurization are used to kill microbes in food.
iii. National Medical Laboratory Week. "The microbiome & aging: How the microbes that live on and in you conspire against you." April 26, 2018.
iv. Amica Retirement Community. Hamilton, ON. "Why are older adults more susceptible to pneumonia and respiratory infections?"
v. Public outreach event at Amica Retirement Community. "Age, infection and inflammation". July 12,2016.
vi. PROSPECT trial mechanistic sub-study retreat. "What are endotoxins and cytokines?" June 9, 2014.
vii. Lung Association Webinar. "A Breath of Fresh Air: Preventing Pneumonia in Older Adults". April 14, 2014.

Topic: Immunology

Text Interviews:

i. Brighter World, McMaster University. May 26, 2023. The right vaccine at the right time protects older adults. Link
ii. CHATELAINE. April 6, 2023. Is Immunity Debt Real? Two Experts Debunk The Health Phenomenon. Link
iii. The Record. March 23, 2023. Vaccine uptake falters after 'huge misstep in our public communication'. Link
iv. The Globe and Mail. March 10, 2023. Why isn't everyone being offered a COVID booster this spring? Link
v. McGill University. February 10, 2023. Does COVID-19 Mess with the Immune System? Link
vi. CBC News & syndicates. February 8, 2023. COVID-19 vaccination during pregnancy helps protect newborns, Canadian study suggests. Link
vii. Toronto Star. February 7, 2023. COVID-19 vaccine uptake plunges in Canada. Link
viii. The Globe and Mail. January 20, 2023. Does COVID-19 disrupt the immune system? Link
ix. The Bobr Times. January 8, 2023. A new type of vaccine could curb COVID-19. Link
x. Global News. January 7, 2023. A mucosal vaccine could be a COVID-19 game-changer. So why doesn't Canada have one? Link
xi. CTV News. December 20, 2022. Managing your child's asthma during flu season is easier with these resources: experts. Link
xii. Ottawa Citizen & syndicates. December 16, 2022. The viral immunity debate has no simple answer — but masks make a difference, says researcher. Link
xiii. Eminetra. November 16, 2022. Will mask mandates doom us to a perpetual pandemic by slowing children's immunity? Link
xiv. CityNews & syndicates. September 1, 2022. Canada approves Moderna's Omicron COVID vaccine. Link
xv. Best Health. August 15, 2022. Should You Get Another COVID Vaccine? Link
xvi. The Hamilton Spectator. July 28, 2022. Which three doses protect older people better: Moderna or Pfizer? McMaster study finds Moderna more effective in long-term-care. Link
xvii. McKights Long-Term Care News. July 28, 2022. Moderna vaccine better at protecting long-term care residents: study. Link
xviii. Newswise. July 26, 2022. Moderna vaccines better protect long-term care home residents. Link
xix. The Toronto Star. July 14, 2022. Should you get a fourth COVID vaccine dose now or in the fall? Five experts weigh in. Link
xx. The Hamilton Spectator. June 28, 2022. What do we know and don't about COVID-19 vaccines. Link
xxi. CBC News. June 21, 2022. Some Quebecers should get periodic COVID-19 boosters, province's vaccine committee says. Link
xxii. The Scientist. April 26, 2022. Smallpox vaccine recruits skin bacteria to fight disease. Link
xxiii. Scientific American. Syndicated by the Irish Times, Slate and elsewhere. April 10, 2020. What immunity to COVID really means Link
xxiv. Advisory Board. April 15, 2020. We can't reopen the country without answering these 3 questions. Link
xxv. Canadian Medical Journal. September 7, 2021. What's the evidence for COVID-19 booster shots? Link
xxvi. CBC. January 12, 2022. Quebec recommends 3rd dose for those who've recently had COVID-19. Link
xxvii. CBC. October 28, 2021. The latest on the coronavirus outbreak for Oct. 18. Link
xxviii. National Post & syndicates. August 19, 2021. COVID booster shots are being rolled out for all Americans. What about Canada? Link
xxix. Saltwire & syndicates. August 19, 2021. COVID booster shots are being rolled out for all Americans. What about Canada? Link

xxx. Canadian Medical Association Journal. August 20, 2021. What's the evidence for COVID-19 booster shots? Link
xxxi. Medical Press. August 19, 2021. Study supports third vaccination for seniors. Link
xxxii. Global News. April 12, 2019. Remember to get your vaccine booster - adults can lose immunity as they age. Link
xxxiii. Caledon Enterprise. April 26, 2021. Can grandparents in Peel see their grandkids after their first COVID vaccine shot? Link

Broadcast Interviews:

i. The Province. April 12, 2023. Ageism and the pandemic: How Canada continues to let older adults suffer. Link
ii. CBC Radio Metro Morning. February 2, 2023. A COVID-19 vaccine maker in Quebec is shutting down. Immunologist Dawn Bowdish weighs in on what that means for Canadians. Link
iii. CityNews. December 13, 2022. Why are children across Canada getting so sick this year? Link
iv. Billy Kelly Show on CHML 900. September 1, 2022. COVID-19 booster doses, what is available and how they work.
v. Global News. July 30, 2022. Who should take the second booster shot? Link
vi. CHCH. July 26, 2022. Moderna vaccines better protect long-term care home residents: study. Link
vii. CTV News. April 25, 2022. Experts advise Canadians to consider personal risk before getting 4th COVID vaccine dose. Link
viii. Interview in which I discuss the benefits of vaccination for older adults on Metro Morning podcast, Thu Nov 12, 2015, from CBC Radio Toronto (Highlights). Released: 2015. Track 1. Genre: Podcast. and on London AM 960 The Pulse with Devon Peacock (airdate: Nov 13, 2015). Link

Knowledge Translation:

i. Discussing vaccination decisions in long-term care and retirement homes (with reference to our CITF funded research). January 2022. Link
ii. Series of "Back to Mac" posts on questions around 3rd doses. Link
iii. Newswise. Discussing third doses/boosters with reference to our CITF funded study on waning immunity in long-term care. November 16, 2021. Link
iv. Healthing. Should those in LTC homes get a third COVID shot? Link
v. Insauga. Ontario's move to add third booster for seniors backed by study at McMaster in Hamilton. August 18, 2021. Link
vi. PROSPECT knowledge users meeting (research coordinators, nursing staff, and others). "Immune dysregulation and PROSPECT patient possible impact". April 16, 2019. I contributed to this educational session by explaining our findings on immune dysregulation in critically ill patients and describing how this affects patient outcomes.
vii. Moderator at the Vaccination Policy Forum hosted by the Lung Association- Ontario. The purpose of this event was to provide up-to-date information on adult vaccinations to policy makers, health professionals and community groups. November 27, 2018 Link
viii. Dundas Library. April, 9, 2018. "The Aging Immune System".
ix. Westdale Library. September, 19, 2017. "The Aging Immune System".
x. PROSPECT webinar. June 7th, 2017. "The role of the microbiome in immune dysfunction."
xi. PROSPECT (Probiotics for prevention of infection clinical trial) retreat. "The immunology of frailty and implications in the ICU". October, 3, 2016. Talk for research co-ordinators, nurses and PIs of the PROSPECT clinical trial. Gravenhurst, ON.

xii. Amica Retirement Community. December 12, 2013. "Health & the aging Immune system". With Dessi Loukov.
xiii. Café Scientifique. Canadian Human Immunology Network. April 26th, 2013. Panel speaker. "Vaccines: Truths and Myths". Hamilton, ON.

Topic: Aging & Immunity

Text Interviews:

i. How the microbiome can affect your overall health and its effects on aging. Ep 15, Decoding Healthcare Research. Agora Health. December 2023.
    a. YouTube: https://youtu.be/yhlsiAqnxZM?feature=shared
    b. Spotify: https://open.spotify.com/episode/0flH90zGfa5bXAVoy1ZiiF?si=f3680075df4b4a2a
    c. Amazon Music: https://music.amazon.com/podcasts/2e2f7513-9491-40e9-a9dc-a31859560d1d/episodes/fce86aa3-0d05-4245-ab87-08be6b86323b/decoding-healthcare-research-how-the-microbiome-can-affect-your-overall-health-and-its-effects-on-aging-ep-15
    d. Apple Podcasts: https://podcasts.apple.com/co/podcast/how-the-microbiome-can-affect-your-overall-health-and/id1712499485?i=1000642050180&l=en-GB
ii. PsyPost. July 15, 2022. New research suggests adverse childhood experiences accelerate the biological processes of aging. Link

Knowledge Translation:

i. Science on Tap. March 22, 2019. "Secrets of the Super-Centenarians: What does it take to live a long (and healthy) life?". Hamilton, ON.
    - This was a public lecture (100+ people) organized by the McMaster Graduate Students Union.
ii. Hamilton Association of Arts, Literature and Science. September 10, 2016. "Age, infection and inflammation".

Topic: Lung Health

Knowledge Translation:

i. The Human Book event, "Careers" edition. Halton District School Board. May 4, 2016.
    - At this event I was a "Human Book". Participants could check me out for 20 min intervals and ask me questions about science or research. Media coverage by the radio station Y108. Link
ii. The Cerebral Café – a discussion of Death & Disease. Hamilton, ON with Drs Ellen Badone, Megan Brickley and Ms Rochelle Martin.
iii. Ontario Lung Association – Breathe Gala. January 28th, 2016. I was the MC for the major fundraising event for the Ontario Lung Association. This evening raised >$168,000 to support lung health research in Ontario.
iv. Lobby day in support of Bill 41 "The Lung Health Act". Queen's Park, Toronto, ON. November 30, 2015
    - I, and some of my trainees, met with MPPs including Speaker of the House, MPP Dave Levac on the importance of establishing a Lung Health Act for Ontarians.

Topic: Miscellaneous

Text Interviews:

I. Policy Options - Institute for Research on Public Policy. October 18, 2022. Remote rehabilitation can offer health-care relief, but there are still problems. Link

Knowledge Translation:

I. "Insights into obstacles and challenges in Clinical Research". Career development seminar for clinician scientists in the Division of Respirology. Clinical Research Rounds. The Firestone Institute for Respiratory Health. January 16, 2023.
II. Janssen Future Leaders Program. McMaster University. "How I lead an Academic Research Institute". September 21, 2022.
III. Aldershot High School, I-STEM Program, Human Book Event. November 14, 2018.
    - I was a "human book" where the students could ask me anything about a career in STEM.
IV. McMaster Undergraduate Student Lung Health Club. "Lung health research in the Bowdish lab and across campus". November 13, 2018
V. Donor Appreciation Day. Research participants were invited to the lab to hear a presentation on our progress, meet the trainees who performed the work and have a tour of the lab and facilities. June 19, 2018. Link
VI. Dundas Central School grades 3-5. "Who's got more cooties – boys or girls?". February 22, 2018.
VII. McMaster Women in Science and Engineering. Mentor of the Month. "Speaking as an expert…Finding your scientific voice and using it for the greater good." June 21, 2017.
VIII. McMaster Children and Youth University. "Who's got more cooties, boys or girls?". April 29, 2017.
IX. McMaster Undergraduates in Research Association. "Opportunities for undergraduates in research." December 1, 2016.
X. Lung Association Pop-up Park "Take a Breather" event. "Why I'm Taking a Breather with the Lung Association." November 3, 2016.
XI. Deputation for the Standing Committee on Social Policy on Bill 41 – "The Lung Health Act will benefit older adults." Queen's Park, Toronto, ON. June 6, 2016.
XII. Ontario Lung Association/Firestone Respiratory Health Institute joint presentation & fundraiser. "Introducing the Breathing as One campaign". October,26, 2015
XIII. "Lung Health is Good Health in Older Adults". Presentation hosted by the Lung Association for the Investors Group. August 25, 2015.
XIV. Research2Reality social media campaign. Research2Reality is a groundbreaking initiative that shines a spotlight on world-class scientists engaged in innovative and leading edge research in Canada. This video series is continually updated to celebrate the success of researchers who are establishing the new frontiers of science and to share the impact of their discoveries with the public. February 24, 2015. Link
XV. "Lung Health is Good Health in Older Adults". Presentation to Hamilton Probus club. April 14, 2015.
XVI. "My career path as a scientist". Westdale High School- Career day. Hamilton, Ontario. March 31, 2015.
XVII. Lung Association Breathe Gala. Interview in support of lung health. January 29, 2015.
XVIII. Café Scientifique "Breathing Easy: Lung Health is Good Health for Older Adults". Public event co-sponsored by the Ontario Lung Association and the Canadian Longitudinal Study of Aging. Hamilton, Ontario. November 27th, 2014.
XIX. Ontario Lung Association Board meeting. "Aging and Lung Health – spreading the word." Toronto, ON. October 24, 2014.
XX. McMaster Undergraduates in Research Association (MURSA). "Undergraduate opportunities in Research". October 22, 2014.

XXI. Presentation of donation from Gamma-Dynacare to the Lung Association. “Lung Health is Good Health in Older Adults” July 16, 2014
XXII. MIRC Trainee Association Event. “Choosing a post-doctoral position, in Canada or overseas.” August 12, 2013.
XXIII. FHS Post-doctoral fellow association. “Advice on applying for a faculty position.” June 5, 2013.
XXIV. Presentation for the Hamilton chapter of CAGIS (Canadian Association of Girls in Science). McMaster University. “Good germs/Bad germs.” March 7th 2013.
XXV. Biomedical Leadership conference. University of Guelph. “My career path: From McMaster & back again.” January, 26th, 2013.
XXVI. McMaster Undergraduates in Research Association (MURSA). “Undergraduate opportunities in Research.” October 12, 2012.

This is Exhibit "B" referred to in the Affidavit of Dr. Dawn Bowdish in the City of Hamilton, in the Province of Ontario, before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

Mackenzie Daniel Frederick Faulkner, a Commissioner,
etc., Province of Ontario, while a Student-at-Law.
Expires April 6, 2025.

The Firestone Institute for Respiratory Health

Firestone Institute for Respiratory Health (FIRH)
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March 14, 2024

Re: Assessment of Dr. Byram Bridle's claims

Dear Council,

As per your request, I have read the transcript of Dr. Byram Bridle's radio interview (Transcription of Podcast: https://podcasts.apple.com/ca/podcast/new-peer-reviewed-study-on-covid-19-vaccines-suggests/id1318830191?i=1000523346577), on The Alex Pierson Show and assessed whether the claims he made were valid. I have also read the affidavits you provided to ascertain whether he had provided scientific evidence to back those claims. For background, I am a professor of medicine at McMaster University, the Canada Research Chain in Aging and Immunity and an immunologist specializing in respiratory infections and vaccinations in older adults. During the pandemic, I co-led Canada's largest study of COVID-19 vaccinations and infections in residents of long-term care and retirement homes, as well as studies on COVID-19 vaccine immunogenicity in immunosuppressed individuals, pregnant women and collaborated on studies of Long COVID. Consequently, I am confident that I can assess the veracity of claims about COVID-19 vaccines.

Synopsis: The Spike protein is not toxic and there is no evidence that vaccination results in dangerous levels of inflammation, organ damage, or loss of fertility. There is no reasonable evidence that the Spike protein produced during mRNA vaccination is toxic. These claims appear to arise from misunderstanding or misrepresentation of a few rodent studies studying how the binding of the Spike protein on the virus to the angiotensin-converting enzyme 2 (ACE2) receptor may contribute to pathology during infection. There is no plausible biologic reason that vaccines against COVID-19 would alter fertility or lead to any long-term adverse consequences in breastfed babies of mothers who have been vaccinated. It has been observed that the immune response to vaccines is associated in very rare instances with myocarditis, but that this is not a direct effect of spike protein. Dr. Bridle incorrectly infers that animal studies studying the mechanisms by which *infection* damages the body would also occur during vaccination. This is incorrect and the quality of evidence used to support this assertion is poor and relies on case-studies, his own review studies, or studies with low sample sizes. I have limited my assessment of these claims to studies that were published contemporaneously with

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this interview or to which Dr. Bridle refers to in his affidavit, but these claims have been continually debunked since then.

Claim #1: "…. heart problems, lots of problems with cardiovascular system bleeding and clotting is all sociated with severe COVID-19 and looking and doing that research what has been discovered by scientific community is the spike protein on its own is almost entirely responsible for the damage to cardiovascular system if it gets into circulation."

At the time of the interview, it was well known that COVID-19 was associated with cardiovascular complications. The number of cardiovascular complications (e.g., heart attacks), inflammatory cardiac conditions (e.g., myocarditis and pericarditis) and cerebrovascular events (e.g., stroke) were extremely high in people who survive COVID-19 infections, even those who were not sick enough to require hospitalization. In a large study comparing cardiovascular events in 53,760 individuals who had COVID-19 between March 2020 and January 2021 and two sets of control cohorts (i.e. one control group included 5,637,647 people of the same ethnicity, age and sex in the same time frame and the other included 5,859,411 people from a pre-pandemic time period in order to control for differences in health services delivery or stress caused by the pandemic), it was found that although these events were more likely to occur in people who had severe disease, even mild COVID-19 that did not require medical attention was associated with an increased risk of a cardiovascular event between 30 days and one year after infection[1]. No credible human data was provided to support the claim that this was due to circulating Spike protein. Subsequent studies have found that vaccination protects from these events (Mercadé-Besora N, Li et al. The role of COVID-19 vaccines in preventing post-COVID-19 thromboembolic and cardiovascular complications. Heart. 2024 Mar 12:heartjnl-2023-323483.)

Claim#2: "The spike protein gets into the blood, circulates through the blood in individuals over several days post vaccination. It accumulates, once it gets the blood and accumulates in a number of tissues, such as the spleen, the bone marrow, the liver, the adrenal glands. One that's a particular concern for me is it accumulates at quite high concentrations in the ovaries"

Vaccines are injected into the muscles (most commonly the deltoid muscle in the arm) because there are very few arteries or blood vessels in the injection site. This

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allows resident immune cells to take up the vaccine and transport it through the lymphatics to lymph nodes (small organs with millions of immune cells) where immune responses are initiated.
The lymphatics return fluid to the blood stream and so there is the possibility that some of the vaccine may enter the blood. Safety data submitted to the European Medicines Agency (https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf) shows that about 1% of the vaccine may enter the circulation, where it will be taken up by the liver. This study demonstrates that there is no detectable vaccine after approximately 48 hours.

An important distinction is that the conformation of the Spike protein encoded by the vaccine differs from that on the virus. The Spike protein is on the surface of the virus and binds to the ACE2 receptor (often called ACE2R) on human cells to allow it to enter cells and start infection. The current mRNA vaccines (i.e., Pfizer/Comirnaty, Moderna/SpikeVax), viral vector vaccines (i.e., AstraZeneca/COVISHIELD, J&J[2]), and protein based vaccines (e.g. Novavax) target the Spike protein. After vaccination, local immune cells take up the mRNA, which encodes the sequence of the Spike protein (Figure 1). Importantly, these instructions include an 'anchor sequence', which means that the Spike protein that is produced will be expressed on the surface of the immune cell. Consequently, the Spike protein is 'anchored' to the surface of the immune cell and is not excreted (i.e., it is not excreted from the cell). The decade of pre-clinical (non-human) and early clinical work on related vaccines, such as MERS (Middle-Eastern Respiratory Syndrome), found that expressing the Spike protein on the surface created greater immune responses than if it were to be excreted from the cells so this 'anchor' is a feature of all the mRNA (Pfizer/Moderna) and adenoviral vector (AstraZeneca/J&J) vaccines [3]. In order for the *virus* to bind to the ACE2 receptor and enter a cell, the Spike protein must change shape. The Spike protein is normally in a 'closed' conformation but when it encounters the ACE2 receptor it becomes 'open' (also called the 'fusion' conformation). The mRNA instructions used in the mRNA vaccines encode the 'closed' formation of the Spike protein, and consequently the Spike protein that is produced by the vaccine cannot bind to the ACE2 receptor and cannot cause any of the health issues that occur when the virus binds the ACE2 receptor as the Spike protein 'opens'. Note that in contrast to the other vaccines, the AstraZeneca/COVISHIELD/Vaxzevria vaccine encodes the 'open' version, but it remains attached to the cell and there is no evidence that this binds ACE2 receptor.

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The study that Dr. Bridle refers to (" they found the spike protein in circulation, so in the blood of 11 of those 13 healthcare workers that had received the vaccine." found circulating Spike protein occurring after vaccination found that it was at vanishingly low levels[4] (<90 pg/ml or 0.00000000009 g; 100,000 x lower than any of the concentrations used in studies showing any toxicity). It is believed that a miniscule amount of the Spike protein that is anchored to immune cells may be found in the blood because proteases (enzymes that digest protein) in our blood randomly digest proteins, including those that cover our immune cells and some of those proteins end up in the circulation. This natural process of digestion means that many proteins that are normally expressed on cells are found at these low concentrations in the blood. Crucially, this vanishingly low level of detected Spike protein is in the closed conformation so it would not be able to bind to the ACE2 receptor[4].

Dr. Bridle's assertion that the Spike protein accumulates in the ovaries is not consistent with the documents he has provided and has never been supported in the literature. He cites a document written in Japanese which is frequently circulated as showing evidence that the Spike protein from vaccines accumulate in the ovaries. This document is not in the peer-reviewed literature and its origin is unclear. Because the origin of this document is unclear, it has not been critiqued in the peer-reviewed literature, and I cannot verify the authenticity of the English translation available on various websites, I do not consider it to be a reliable source on biodistribution; however, I will address what the document does and does not say. First, it is important to realize that this study (and other biodistribution studies) do not measure the mRNA or the Spike. This report (and other biodistribution reports) measures the lipid nanoparticles (LNP) that carry the mRNA (sometimes called the 'lipid shell'). Another important point to keep in mind is that lipids dissolve lipids (taught as 'like dissolves like' in high-school chemistry) so the lipid shell and the lipid dye used to label it (Cholesteryl-1,2-3H(N)]-Cholesteryl Hexadecyl Ether) will have an affinity for lipid rich areas of the body. Unlike human ovaries, rodent ovaries are covered with a thick layer of fat and are thus more likely to accumulate lipids such as the dye used in the study so the estimate of accumulation in humans is likely even lower. Although the integrity of this document is dubious, the distribution of the lipid nanoparticles is similar to other biodistribution studies of lipid nanoparticles[5, 6].

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Claim #3 "we have known for a long time that the spike protein is a pathogenic protein. It is a toxin"

Dr. Bridle did not submit any supporting documentation to support this claim and I do not know of any. Some affadavits included speak to experiments where researchers found that if they mixed spike protein with cultured immune cells they might be an inflammatory response, but this is not unusual, and does not constitute a protein being a toxin.

Claim #4: " When in circulation, the spike protein can bind to the receptors that are on our platelets and the cells that line our blood vessels. When that happens, it can do one of two things. It can either cause platelets to clump and that can lead to clotting. That's exactly why we've been seeing clotting disorders associated with these vaccines. It can also lead to bleeding and of course with the hearts involved, it's part actually part of the cardiovascular system."

Dr. Bridle has not submitted evidence to support this claim. He appears to be referencing Vaccine Induced Thromobotic Thrombocytopenia (VITT), which is a rare clotting disorder which can occur after vaccination with a viral vector vaccine. People who develop VITT seem to have pre-existing antibodies to a protein required for clotting called PF4 (platelet factor 4) and for reasons that we do not understand, these antibodies seem to be triggered by the viral vector and can start a dangerous clotting cascade[7]. A key point is that these antibodies seem to be generated in response to the viral vector (i.e., the virus that is used in the AZ and J&J vaccines), but NOT the Spike protein (which is made after vaccination by both the mRNA and viral vector vaccines). Once the mechanism of this was discovered, treatments were quickly developed and fatalities are now exceedingly rare; however, since this appears to be a feature of the viral vector, there will need to be extensive monitoring of any vaccine using viral vectors[8, 9]. There have been rare cases of clotting disorders after administration of mRNA vaccines, often in people who have antibody disorders, but they do not occur at rate that is higher in the weeks after mRNA vaccination than at other times, so that it is very unlikely that it is unclear as to whether these are initiated by the Spike protein[10]. For this reason, clotting disorders are not listed as an adverse event possibly associated with mRNA vaccines by vaccine regulatory agencies.

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In his affidavit Dr Bridle cites the work of my McMaster colleagues (Appelbaum J, Arnold DM, Kelton JG, Gernsheimer T, Jevtic SD, Ivetic N, Smith JW, Nazy I. SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia. Blood Adv. 2022 Apr 12;6(7):2250-2253. doi: 10.1182/bloodadvances.2021005050.) as support that the Spike protein can cause clotting and as evidence that the Spike protein can be found in the blood of vaccinated people. It is important to note that this is an extremely unusual case that has not been replicated and it was published a year after the radio interview, meaning that it could not be the basis on which Dr Bridle made these claims.

Dr. Bridle did not submit any reputable scientific documents on his claim that the Spike protein also causes bleeding and that causes cardiovascular issues.

Claim #5: "Indeed, if you inject the, the purified spike protein into the blood of research animals, they get all kinds of damage to cardiovascular system, it can cross the blood brain barrier and cause damage to the brain." And "The protein that can also cross the blood brain barrier and cause neurological damage. That's why also in the fatal case of the blood clots, many times it's seen in the brain"

I did not see any support for this claim. It was known at the time that COVID infection was associated with strokes (i.e. clots that affect the brain)[1]. I suspect he was referring to an animal study by Rhea et al,[11] that he references in his "Vaccinations in children". This is a mouse study in which high levels of radiolabelled Spike protein (or more specifically, the S1 subunit that binds the ACE2R because it is in the 'open' position) were injected to determine if it could cross the barrier between blood and brain. The authors were very clear in the manuscript that they were attempting to mimic the high amounts of Spike protein that would occur during an *infection* (hence using the open conformation) and *not* vaccination[11]. Importantly, they did not report any neurologic issues with the mice. For context, all the food and nutrients that the brain needs are transported through the blood, so it is not unusual for a protein to enter the brain. The researchers did not find that the mice had any neurologic issues as a result of the Spike protein entering the brain so there is no reason to be concerned that the Spike protein alone causes brain damage. There has been no evidence of neuroinflammation in vaccinated individuals; however, there is evidence of brain inflammation causing damage[12, 13] and cognitive decline after infection with the SARS-CoV-2 virus[14].

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Claim #6: " they were trying to show that the antibodies from the vaccine get transferred through breast milk and the idea was this may be a good thing because it would confer some passive protection to babies. However, what they found inadvertently was that the the messenger in vaccines actually get transferred through the breast milk for delivering the vaccine vector itself into infants that are breastfeeding also with this, now we know the spike protein gets into circulation any proteins in the blood will get concentrated in breast milk looking into the adverse event database in the United States, we have found evidence of suckling infants experiencing bleeding disorders in the gastrointestinal tract."

Dr. Bridle claims that this reference supports this statement (Fu W, Sivajohan B, McClymont E, Albert A, Elwood C, Ogilvie G, Money D. Systematic review of the safety, immunogenicity, and effectiveness of COVID-19 vaccines in pregnant and lactating individuals and their infants. Int J Gynaecol Obstet. 2022 Mar;156(3):406-417. doi: 10.1002/ijgo.14008.); however, this does not look at mRNA in breastmilk but rather reviews studies on vaccine safety and adverse events and finds they are the same in pregnant women.

He cites this paper "Low, J.M., Gu, Y., Ng, M.S.F., Amin, Z., Lee, L.Y., et al. (2021) Codominant IgG and IgA expression with minimal vaccine mRNA in milk of BNT162b2 vaccinees. NPJ Vaccines. 6(1):105. doi:10.1038/s41541-021-00370-z". For context, the study did not find mRNA in all mothers and in those it was found, it was at such low levels that it was difficult to detect. In order to find it the authors had to purify all the mRNA from the breastmilk (this would include mRNA from food, and the mother's cells) and then amplify that mRNA in the lab. From that enriched sample, only 4 participants had detectable mRNA from the vaccine and it was so low that it was barely above the level of detection. Consequently, the authors concluded that the amount of vaccine mRNA was exceedingly low in breastmilk. Even if mRNA were to transfer, it is important to remember that there is a lot of mRNA in breastmilk (from the cells that the mother transfers) as there is in all our food (all vegetables and animals have mRNA in their cells) and this is destroyed by the digestive system. There is no credible reason to be concerned about this. Indeed, the authors' conclusion was that the amount of transfer of mRNA was vanishingly low but that the mothers did transfer high quality antibodies that would be expected to protect babies through the breastmilk. These results were confirmed in the second study (Yeo, K.T., Chia, W.N., Tan, C.W., Ong, C., Yeo, J.G., et al. (2022) Neutralizing activity and SARSCoV-2 vaccine mRNA persistence in serum and breastmilk after BNT162b2 vaccination in

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lactating women. Front Immunol. 12:783975. doi:10.3389/fimmu.2021.783975) where mRNA was found at vanishingly low levels in 2% of the lactating women.

There is no credible evidence that this is cause for concern.

Claim #7: "Canadian blood services are saying people that who have been vaccinated can donate. We don't want transfer of these pathogenic spike proteins to fragile patients for being transfused with that blood."

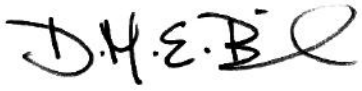
Dr. Bridle did not submit any supporting evidence for this claim.

Claim #8 "one of my questions is, will we be rendering young people infertile? Some of them infertile? "

Dr. Bridle did not provide any support for this claim in his affidavit. At the time this claim was made there was already work done investigating fertility. The largest study at the time included more than 2000 couples trying to conceive, found that vaccination did not impair conception (although COVID-19 infection was associated with a transient decrease in male fertility)[15]. In addition, despite the fact that women are supposed to be practicing multiple methods or birth control during trials of new vaccines and medications, there were 57 accidental pregnancies in the clinical vaccine trials for Pfizer/BioNTech, Moderna, and AstraZeneca, and there was no difference in the number of miscarriages or other adverse pregnancy outcomes from the expected rate[16]. Other studies found no change to ovarian follicular function after vaccination[17]. Having antibodies to the Spike protein either due to infection or vaccination was not associated with decreased fertility[18]. Since this time multiple studies have shown no association with infertility.

Collectively these claims were not supported by data.

Sincerely,



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References:

1. Xie, Y., et al., *Long-term cardiovascular outcomes of COVID-19.* Nature Medicine, 2022.
2. Bos, R., et al., *Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses.* npj Vaccines, 2020. **5**(1): p. 91.
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12. Rutkai, I., et al., *Neuropathology and virus in brain of SARS-CoV-2 infected non-human primates.* Nature Communications, 2022. **13**(1): p. 1745.
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17. Bentov, Y., et al., *Ovarian follicular function is not altered by SARS–CoV-2 infection or BNT162b2 mRNA COVID-19 vaccination.* Human Reproduction, 2021. **36**(9): p. 2506-2513.
18. Morris, R.S., *SARS-CoV-2 spike protein seropositivity from vaccination or infection does not cause sterility.* F&s Reports, 2021. **2**(3): p. 253-255.

This is Exhibit "C" referred to in the Affidavit of Dr. Dawn Bowdish in the City of Hamilton, in the Province of Ontario, before me at the City of Toronto in the Province of Ontario, on March 15, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

Mackenzie Daniel Frederick Faulkner, a Commissioner,
etc., Province of Ontario, while a Student-at-Law.
Expires April 6, 2025.

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

ACKNOWLEDGMENT OF EXPERT'S DUTY

1. My name is Dawn Bowdish. I live in the City of Hamilton, in the Province of Ontario.

2. I have been engaged by or on behalf of the Defendant, David Fisman, to provide evidence in relation to the above-noted court proceeding.

3. I acknowledge that it is my duty to provide evidence in relation to this proceeding as follows:

   (a) to provide opinion evidence that is fair, objective and non-partisan;

   (b) to provide opinion evidence that is related only to matters that are within my area of expertise; and

   (c) to provide such additional assistance as the Court may reasonably require, to determine a matter in issue.

4. I acknowledge that the duty referred to above prevails over any obligation which I may owe to any party by whom or on whose behalf I am engaged.

March 15, 2024

Date

D.M.E.B

Signature

NOTE: This form must be attached to any expert report under subrules 53.03(1) or (2) and any opinion evidence provided by an expert witness on a motion or application. RCP-E 53 (July 22, 2014)

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
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PROCEEDING COMMENCED AT TORONTO

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DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

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PROCEEDING COMMENCED AT TORONTO

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ourt File No. CV-22-00691880-0000

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B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

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Defendants

AFFIDAVIT OF DR. BRIAN CONWAY

I, Dr. Brian Conway, of the City of Vancouver, in the Province of British Columbia, MAKE OATH AND SAY:

1. I have been asked by counsel to the defendant, Dr. David Fisman, to provide an expert opinion on the claims expressed on the COVID-19 vaccine safety and efficacy by Dr. Byram Bridle in June 2021 as part of this litigation. I am an internal medicine physician, specializing in infectious diseases, licenced to practice in the Province of British Columbia, and, as such, have knowledge of the matters contained in this Affidavit. A copy of my CV is attached hereto as **Exhibit "A"**.

2. Attached hereto as **Exhibit "B"**, is a copy of my Expert Report dated March 15, 2024.

3. Attached hereto as **Exhibit "C"** is an executed copy of my Acknowledgment of Expert's Duty Form 53 dated March 15, 2024.

SWORN by Dr. Brian Conway of the City of Vancouver, in the Province of British Columbia, before me at the City of Toronto, in the Province of Ontario, on March 18 2024 in accordance with O. Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits
(or as may be)

DR. BRIAN CONWAY

JAAN LILLES

This is Exhibit "A" referred to in the Affidavit of Dr. Brian Conway in the City of Vancouver, in the Province of British Columbia before me at the City of Toronto in the Province of Ontario, on March 18, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

CURRICULUM VITAE
DR. BRIAN CONWAY

Last updated:

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Brian Conway, MD, FRCPC

President and Medical Director
Vancouver ID Research & Care Centre Society
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Tel: (604) 642-6429
Fax: (604) 642-6419
Email: brian.conway@vidc.ca

POST-SECONDARY EDUCATION

University or Institution	Degree	Subject Area	Dates
McGill University	MDCM	Medicine	1977-82
McGill University Queen Elizabeth Hospital	Mixed Internship	General Medicine	1982-83
McGill University Royal Victoria Hospital	Residency Training	Internal Medicine	1983-86
University of Manitoba	Specialty Fellowship	Infectious Diseases	1986-88
Harvard University	Post-Doctoral Fellowship	HIV/AIDS	1988-90

SPECIAL PROFESSIONAL QUALIFICATIONS

1983: National Board of Medical Examiners (272338)
1983: Medical College of Canada (55273)
1986: American Board of Internal Medicine (11388)
1986: Royal College of Physicians and Surgeons of Canada, Internal Medicine (363372)
1987: Corporation Professionnelle des Médecins du Québec, Internal Medicine (83417)

EMPLOYMENT RECORD

University, Company or Organization	Rank or Title	Dates
University of Ottawa	Assistant Professor Department of Medicine	July 1990 – June 1994
University of Ottawa	Assistant Professor Department of Microbiology & Immunology	July 1990 – June 1994
Children's Hospital of Eastern Ontario	Consultant Virologist	July 1992 – June 1994
University of British Columbia	Assistant Professor Department of Medicine	July 1994 – June 1998
British Columbia Center for Excellence in HIV/AIDS	Head, Clinical Retrovirology	July 1994 – June 1998

British Columbia Centre of Excellence for HIV/AIDS	Chairman, Therapeutics Guidelines Committee	July 1994 – June 1998
St-Paul's Hospital	Associate Director Immunodeficiency Clinic	July 1994 – June 1998
University of British Columbia	Assistant Professor (Part-Time) Department of Pharmacology & Therapeutics	July 1998 – June 2000
Viridae Clinical Sciences Inc	Medical Director	July 1998 – June 2000
University of British Columbia	Assistant Professor (Full-Time) Department of Pharmacology & Therapeutics	July 2000 – July 2004
University of British Columbia	Associate Professor (Full-Time) Department of Pharmacology & Therapeutics	July 2004 – October 2009
University of British Columbia	Professor (Full-Time) Department of Pharmacology & Therapeutics	November 2009 – December 2012
Downtown Infectious Diseases Clinic	Medical Director	July 2000-April 2012
Vancouver Coastal Health Authority Pender Community Health Center	Infectious Diseases Consultant	July 2000 – September 2012
CoolAid Community Health Centre	Infectious Diseases Consultant	August 2007– September 2008
Vancouver ID Research and Care Centre Society	Medical Director	April 2012 – May 2015
Vancouver ID Research and Care Centre Society	President and Medical Director	June 2015 – Present
Simon Fraser University	Adjunct Professor	January 2021 - Present

TEACHING

Areas of Special Interest and Accomplishments

From 2000-2005, I was responsible for the week on Immunodeficiency in the Host Defenses and Immunity (HDI) block in the first year of the undergraduate medical curriculum. I delivered four lectures within this week and led an interactive, two-hour case discussion section. As a result of the success of this week, I was asked to take a leadership role in HIV teaching in the school of Pharmacy, as well as in the Departments of Pathology and Pharmacology & Therapeutics, and in the Experimental Medicine graduate program. Within the medical school expansion, the week I chaired was selected as the demonstration week that will be used for the development of the multi-site medical school curriculum for the University of British Columbia. This allowed us to pilot a number of teaching modalities (including the multi-site case review model) that has been incorporated into the medical curriculum on an ongoing basis.

In July 2004, I was selected as course director for the Doctor Patient and Society (DPAS 410) course for the first year of the medical curriculum. The initial appointment was for a period of 3 years and involved special responsibility for the distribution of the program to the new Northern Medical Program (NMP) in Prince George and the Island Medical Program (IMP) in Victoria. I was reappointed for a second term in 2007. The overall course evaluations were the best in memory, due to our approach to integrate DPAS into the overall curriculum and harmonize its objectives with those of other courses. Further, we incorporated reflective writing and other participatory activities into the course to enhance its relevance and its ability to reflect core competencies for graduation. The students and other educators reviewed these changes quite favorably.

Session	Course Number	Scheduled Hours	Class Size	Hours Taught			
				Lectures	Tutorial	Labs	Other
1994-1996	Med 2[(1)]	14/year	4-8	0	14	0	0
1994-2004	MEDI 501	4/year	20	4	0	0	0
1996-2012	PATH 521	1/year	10	1	0	0	0
1997	MEDI 580[(2)]	30	8	6	2	0	22
1997-1998	Med 1	2/year	160 – 240	2	0	0	0
1998-2012	PCTH 300/305	2/year	40	2	0	0	0
1999	Med 1	32	8/160	2	30	0	0
2000-2012	Med 1	74/year	8/160	4	30	0	40[(3)]
2000-2012	PCTH 404	4/year	7	4	0	0	0
2000-2012	PCTH 500	4/year	10	4	0	0	0
2002-2012	PHAR 480	9/year	80	5	4	0	0
2003-2012	PHAR 550	2/year	20	3	0	0	0
2003-2007	PATH 417[(4)]	22/year	12	0	0	0	22
2004-2012	DPAS 410[(5)]	90/year	240	12	90	0	0
2005-2007	PHAR 352	3/year	50	2	0	0	0

(A) Courses taught at the University of British Columbia (UBC)

(1) : Small group teaching in Infectious Diseases

(2) : In addition to responsibilities as a lecturer, I acted as overall course coordinator

(3) : From 2000-2002, I was the coordinator for the Immunodeficiency week in the Host Defense And Immunity (HDI) block within the first year of the medical curriculum. In addition to my work as a lecturer and a small group tutor, I devoted an additional 40 hours to preparation of the week, the weekly quiz, setting of the examination and other tasks. For 2003-2004, when this week was been selected as a demonstration week for the development of the multi-site medical school curriculum for the University of British Columbia, my time spent on this project significantly expanded.

(4) : Distance learning course on bacterial pathogenesis, for which I was the associate coordinator: The time commitment was 2 hours/week for an 11-week block.

(5) : Course Instructor

(B) Graduated students supervised

Student Name	Program Type	Subject	Year Start	Year Finish
Ying Dong Xu	PhD Experimental Medicine	Effects of Gamma Irradiation on HIV	1993	2000
Onesmo Mpanju	PhD Experimental Medicine	Antiretroviral Activity of LiGLA	1993	2001
Joyce Msuya	MSc Immunology	Diagnosis of CMV Infection in Advanced HIV Disease	1993	1996
Christine Marshall[(1)]	MSc Experimental Medicine	Virology of Pediatric HIV Infection	1994	2001
Jennie Prasad	MSc Experimental Medicine	Pediatric HIV Infection	1996	1999
Ian Woo	MSc Experimental Medicine	Primary HIV Infection	1996	1999

Harout Tossonian	PhD Pharmacology & Therapeutics	Treatment of HIV infection in IDUs	2003	2009
Jason Grebely	PhD Pharmacology & Therapeutics	Treatment of HCV infection in IDUs	2003	2007
Ahmed Barishee	MSc Pharmacology & Therapeutics	Spontaneous Clearance of HCV in IDUs	2007	2010
Jesse Raffa	MSc Statistics	Adherence Thresholds to Antiretroviral Drugs in IDUs	2004	2006

(1) : Recipient, Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) student travel award, 1996 ($750)
(2) : Recipient, University of British Columbia UGF Award ($7,500)
(3): Recipient, University of British Columbia UGF Award ($6,000)
(4): Recipient, Young investigator Award, Clinical Track, Canadian Association for HIV Research, 2004 ($2,000)

(C) Post-doctoral fellows supervised

Student Name	Program Type	Subject	Year Start	Year Finish
Douglas Manion[(1)]	Infectious Diseases Fellowship	Concepts in HIV and CMV Viral Load	1992	1994
Rani Azar	Infectious Diseases Fellowship	Evaluation of Viral Load in HIV-Infected patients	1994	1995
Fahad Al Rabiah	Infectious Diseases Fellowship	HIV and HCV Co-Infection	1995	1996
Danielle Rouleau[(1, 2)]	Infectious Diseases Fellowship	Primary HIV Infection	1995	1997
Valentina Montessori [(2)]	Infectious Diseases Fellowship	Primary HIV Infection	1995	1997
Adel Al Othman	Infectious Diseases Fellowship	Efficacy of a novel formulation of saquinavir	1996	1997
John Duncan [(3)]	Medical Residency Research	Facial Nerve Paralysis: Atypical presentation of HIV Seroconversion	1996	1997
Bruce Hiller[(4)]	Medical Residency Research	A Decision-Analysis Approach to the Selection of Antiretroviral Therapy	1996	1997
Khalid Al Habib	Medical Residency Research	*R. dentocariosa* Bacteremia	1997	1998

(1) : Recipient, Canadian HIV Trials Network Fellowship ($45,000/year, 2 years)
(2) : Recipient, Canadian Society for Clinical Investigation Trainee Award (1996, $750)
(3): Medical Residents Research Day, Best Case Presentation (1997)
(4): Medical Residents Research Day, Best Overall Presentation (1997)

(D) Other students supervised

Student Name	Program Type	Subject	Year Start	Year Finish
Marcus Shaw[(1)]	Bachelor Medical Sciences Program	HIV Drug Resistance Testing	1990	1992
Deepali Kumar[(1)]	Bachelor Medical Sciences Program	Evaluation of LiGLA as an Antiretroviral Agent	1991	1993
Jennifer Manning	University of Victoria BSc Co-Op Research Program	Nevirapine-Based Antiretroviral Therapy	1994	1995

Laura Heemskerk	University of Victoria BSc Co-Op Research Program	HIV Drug Resistance Testing	1994	1995
Sam Mercer	University of Victoria BSc Co-Op Research Program	Ultra-Sensitive PCR for HIV Plasma Viral Load Measurement	1995	1996
Janice Whitney	University of Victoria BSc Co-Op Research Program	Nevirapine-Based Antiretroviral Therapy	1996	1997
David Hall	University of Victoria BSc Co-Op Research Program	Primary HIV Infection	1996	1997
Brad Hedberg	University of Victoria BSc Co-Op Research Program	Acute HIV Infection in IVDUs	1996	1998
Stacey Grubb	U.B.C. BSc Co-Op Research Program	Delavirdine-Based Antiretroviral Therapy	1999	2000
Ken Tang	U.B.C. BSc Co-Op Research Program	Acute HIV Infection	2000	2001
Amanda Roze des Ordons(2)	U.B.C. Faculty of Medicine Summer Student	Acute HIV Infection in IVDUs	2002	2002
Miriam Farah(3)	U.B.C. Faculty of Medicine Summer Student	Evaluation of HIV Teaching in North American Medical Schools	2002	2002
Liana Hwang(4)	U.B.C. BSc Co-Op Research Program	Use of Antiretroviral Therapy in HIV-Infected IVDUs	2002	2003
Jason Grebely(4)	U.B.C. BSc Co-Op Research Program	HIV/HCV Co-Infection in IVDUs	2002	2002
Sharon Aujlay	U.B.C. Faculty of Medicine Summer Student	Development of the Prototypical Week – A Pilot Study for the Distributed Medical Curriculum	2003	2003
Behroz Rashidi	UBC BSc. Directed Studies Student	Atazanavir substitution in HIV infected individuals	2007	2007
Niloofar Estbak	UBC BSc. Directed Studies Student	Time Limited HAART for the treatment of acute/early HIV infection	2008	2008
Maya Tong	UBC BSc. Directed Studies Student	HCV Re-infection in IDUs	2010	2011
Jeffrey Wang	UBC BSc, Directed Studies Student	A pilot, observational, open-label study of Kaletra® and Celsentri® combination therapy for the management of HIV infection in the setting of HCV co-infection	2011	2012
Arshia Alimohammadi(5)	UBC Medical School Student	Various projects on HIV and HCV	2012	2017
Tyler Raycroft(6)	UBC Medical School Student	Various projects on HIV and HCV	2013	2016
Julie Holeksa(7)	Research associate at VIDC; currently completely PhD program at Malmö University, Sweden	Various projects on HIV and HCV	2017	2019

(1) : Pharmaceutical Manufacturers Association of Canada (PMAC) Studentship Award Recipient ($4,000/year, 2 years)

(2) : University of British Columbia Faculty of Medicine Summer Studentship Award Recipient ($3,000)

(3): University of British Columbia Faculty of Medicine Summer Studentship Award Recipient, Undergraduate Education ($2,500)

(4) : NSERC Co-Op Student Award Recipient ($3,500)

(5) : Canadian Network on Hepatitis C – CanHep C Summer Student Fellowship 2015 ($4,500)
(6): Canadian Network on Hepatitis C – CanHep C Summer Student Fellowship 2016 ($4,500)
(7): 2018 EASL ILC Young Investigator Registration Bursary, 2018 EASL HCV Monothematic Conference Young Investigator Full Bursary, 2018 Canadian Liver Meeting Poster of Distinction

(E) Visiting lecturer

May 1996	Clinique L'Actuel AIDS Rounds, Montreal -Use of d4T/3TC Combination Antiretroviral Therapy
Oct 1996	Entretiens Jacques Cartier, Montreal - Primary HIV infection
Feb 1997	Toronto Family Practice AIDS Group - Update on NNRTIs
Jun 2002	New York HIV Update – NNRTIs and Related Topics
Jun 2002	Whitman Walker Clinic, Washington D.C. – HIV in the Inner City
Aug 2002	Brown University ID Rounds, Providence - DOT in HIV-Infected IVDUs
Oct 2002	University of Massachusetts Worcester ID Rounds – Use of NNRTIs in Clinical Practice
Nov 2002	Royal Edinburgh Infirmary ID Rounds – NNRTIs: New Data
Feb 2002	Massachusetts General Hospital ID Rounds – New Data on Once Daily HAART in IVDUs
Feb 2003	Brigham & Women's Hospital ID Rounds, Boston – the 2NN Study: Nevirapine vs. Efavirenz
Apr 2003	Vanderbilt University ID Rounds – Once Daily HAART: A Paradigm Shift in HIV Care
May 2003	Henry Ford Hospital ID Rounds – The Role of NNRTIs in First Line HAART – An Update
Sep 2004	University of Alberta Infectious Diseases Rounds – An Update from the International AIDS Conference, Bangkok 2004
Mar 2005	Vanderbilt Medical Center, ID Rounds – An approach to HIV infection in vulnerable populations
Mar 2005	Albert Einstein Hospital – HIV Update
Mar 2005	St. Luke's-Roosevelt Hospital – HIV Update
Jun 2005	University of Texas – Nevirapine vs. Efavirenz
Jun 2005	Miriam Hospital, Brown University – Directly observed therapy
Aug 2005	Harvard University – Tipranavir Update
Apr 2006	University of Manitoba, HIV Care Group – Simplified ARVs: A model for the inner city
Jun 2006	Mt. Sinai Hospital – Update on HCV in vulnerable populations
Feb 2007	Maple Leaf Medical Clinic HIV Rounds – Post AIDS 2006 Forum
Apr 2008	Montreal- Long-term efficacy of HAART in vulnerable populations: Virologic and clinical considerations
Jun 2008	Saskatoon HIV treating physicians – HIV Update

SCHOLARLY AND PROFESSIONAL ACTIVITIES

Areas of special interest and accomplishments

1. During my fellowship at Harvard, I published an article to demonstrate that cultured HIV was distinctly different from the initial in vivo isolate from the infected host. This led to a strategy to sequence isolates directly from mononuclear cells in the host and use PCR techniques that were then being developed to amplify and study these original isolates. In doing this work, I was also involved in the development of the first quantitative PCR assays for the measurement of HIV in infected cells and biological fluids. The tests I helped developed were the first versions of the HIV plasma viral load test that remains a cornerstone of HIV care to this day.
    a. Conway B, Adler KE, Bechtel LJ, Kaplan JC, Hirsch MS. Detection of HIV-1 DNA in crude cell lysates of peripheral blood mononuclear cells by the polymerase chain reaction and non-radioactive oligonucleotide probes. *J Acq Imm Def Synd* 3:1059-1064, November 1990.

b. Kusumi K, Conway B, Cunningham S, Berson A, Evans C, Iversen AKN, Colvin D, Gallo MV, Coutre S, Shpaer EG, Faulkner DV, de Ronde A, Volkman S, Williams C, Hirsch MS, Mullins JI. Human immunodeficiency virus type 1 envelope gene structure and diversity in vivo and following short-term co-cultivation in vitro. *J Virol* 66:875-885, 1992.
c. Conway B, Bechtel LJ, Adler KA, D'Aquila RT, Kaplan JC, Hirsch MS. Comparison of spot-blot and microtiter plate methods for the detection of HIV-1 PCR products. *Mol Cell Probes* 6:245-249, 1992.
d. Conway B, Montpetit M, Raboud J, Salas T, Dufour D, Montaner JSG, O'Shaughnessy MV. Potential applications of proviral load measurement in clinical retrovirology. *J Acq Imm Def Synd* 10(Suppl 2):S45-50, 1995.

2. Later in my career, I became more interested in the development of HIV drug resistance. I was asked to participate in the programs for the evaluation of the clinical significance of drug resistance mutations, and was the only Canadian researcher asked to participate in this effort. Our group was also the first to describe primary lamivudine resistance in clinical isolates. As an extension of this, we were very much involved in the study of primary HIV drug resistance in general, and how it could influence the choice of initial therapy among those who were recently infected. In a landmark study of nevirapine-based therapies, we studied the evolution of drug resistance in the first generation of triple drug regimens including this class.
   a. Hirsch MS, Conway B, Richman D, Brun-Vézinet F, Clotet B, D'Aquila, et al. Antiretroviral drug resistance testing in adults with HIV infection. *JAMA* 279:1984-1991, 1998.
   b. Conway B, Montessori V, Rouleau D, Montaner JS, O' Shaughnessy MV, Fransen S, Shillington A, Weislow O, Mayers DL. Primary lamivudine resistance in acute/early human immunodeficiency virus infection. *Clin Infect Dis* 28:910-911, 1999.
   c. Conway B, Wainberg MA, Hall D, Harris M, Reiss P, Cooper D, Vella S, Curry R, Robinson P, Lange JMA, Montaner JSG. Development of drug resistance in patients receiving combinations of zidovudine, didanosine and nevirapine. *AIDS* 15:1269-1274, 2001.
   d. Little SJ, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, Koup RA, Mellors JW, Connick E, Conway B, Kilby M, Wang L, Whitcomb JM, Hellmann NS, Richman DD. Antiretroviral-drug resistance among patients recently infected with HIV. *N Engl J Med* 347:385-394, 2002.

3. Another great area of interest was acute/early HIV infection itself. One of my trainees, Dr. Montessori, described the clinical characteristics of primary HIV infection in different populations. Wewere able to participate in a number of international efforts in this field. I received a grant to lead oneof the three seminal clinical trials in this field, to evaluate the hypothesis that treatment in the settingof acute/early HIV infection would be uniquely beneficial. Not only did we publish the results of this study, but I wrote one of the key editorials on this subject. It was an important part of my scientific career to have played such a critical role in this field.
   a. Montessori V, Rouleau D, Raboud J, Rae S, Woo I, Montaner JS, Conway B. Clinical characteristics of primary HIV infection in injection drug users. *AIDS* 14:1868-1870, 2000.
   b. Hecht F, Wang L, Collier A, Little S, Markowitz M, Margolick J, Kilby J M, Daar E, Conway B, Holte S. A multicenter observational study of the potential benefits of initiating combination antiretroviral therapy during acute HIV infection. *J Infect Dis* 194:725-733, 2006.
   c. Tossonian H, Conway B. Recent HIV-1 infection: to treat or not to treat, that is the question. *J Infect Dis* 205:10-2, 2012.
   d. Margolick JB, Apuzzo L, Singer J, Wong H, Lee T, Gallant JE, El-Helou P, Loutfy MR, Rachlis A, Fraser C, Kasper K, Tremblay C, Tossonian H, Conway B. A randomized trial of time-limited antiretroviral therapy in acute/early HIV infection. *PLOS ONE* 10:e0143259, 2015.

4. We developed a number of systems of care for the diagnosis and treatment of HIV and HCV infection in vulnerable populations. We were the first to show that co-administration of HIV medications along with methadone in people actively using street drugs would lead to high levels of virologic

suppression. We then developed a more integrated multidisciplinary program to address the needs of this population in an cohesive manner.

a. Conway B, Prasad J, Reynolds R, Farley J, Jones M, Jutha S, Smith N, Mead A, DeVlaming S. Directly observed therapy for the management of HIV-infected patients in a methadone program. *Clin Infect Dis* 38(Suppl 5):S402-8, 2004.
b. Tossonian H, Raffa J, Grebely J, Trotter B, Viljoen M, Mead A, Khara M, McLean M, Duncan F, Fraser C, deVlaming S, Conway B. Methadone dosing strategies in HIV-infected injection drug users enrolled in a directly observed therapy program. *J Acquir Immune Defic Syndr* 45:324-7, 2007.
c. Raffa J, Grebely J, Tossonian H, Wong T, Viljoen M, Khara M, Mead A, McLean M, Duncan F, Petkau AJ, deVlaming S, Conway B. The impact of ongoing illicit drug use on methadone adherence in illicit drug users receiving treatment for HIV in a directly observed therapy program. *Drug Alcohol Depend* 89:306-309, 2007.
d. Conway B, Tossonian H. Comprehensive approaches to the diagnosis and treatment of HIV infection in the community: Can "Seek and Treat" really deliver? *Curr Infect Dis Rep* 13:68-74, 2011.

5. Most recently, we have developed new approaches to the diagnosis and treatment of HIV and HCV infections in vulnerable populations. We have treated the largest number of active injection drug users for HCV in the world and have developed a multidisciplinary approach to engage patients in care and reduce the rate of post-treatment HCV re-infection. The trainee involved in this project, Jason Grebely, has gone on to become the deputy director of HCV programs in Australia. As a result of our programs, I have been a senior investigator on many pivotal HCV studies, and have been a senior/first author on multiple publications, including the MALACHITE study in *Journal of Hepatology*.
   a. Grebely J, Raffa JD, Meagher C, Duncan F, Genoway KA, Khara M, McLean M, Mead A, Viljoen M, DeVlaming S, Fraser C, Conway B. Directly observed therapy for the treatment of hepatitis C virus infection in current and former injection drug users. *J Gastroenterol Hepatol* 22:1519-25, 2007.
   b. Dore GJ, Conway B (co-first author), Luo Y, Janczewska E, Knysz B, Liu Y, Podsadecki T. Efficacy and safety of ombitasvir/paritaprevir/r and dasabuvir compared to IFN-containing regimens in genotype 1 HCV patients: The MALACHITE-I/II trials. *J Hepatol* 64:19-28, 2016.
   c. Grebely J, Dore GJ, Alami NN, Conway B, Dillon JF, Gschwantler M, Felizarta F, Hezode C, Tomasiewicz K, Fredrick LM, Dumas EO, Mensa FJ. Safety and efficacy of glecaprevir/pibrentasvir in patients with chronic hepatitis C genotypes 1–6 receiving opioid substitution therapy. *Int J Drug Policy* 66:73–9, 2019.
   d. Foster GR, Dore GJ, Wang S, Grebely J, Sherman KE, Baumgarten A, Conway B, Jackson D, Asselah T, Gschwantler M, Tomasiewicz K, Aguilar H, Asatryan A, Hu Y, Mensa FJ. Glecaprevir/pibrentasvir in patients with chronic HCV and recent drug use: An integrated analysis of 7 phase III studies. *Drug Alcohol Depend* 194:487–94, 2019.

(A1) Clinical study involvement

Granting Agency	Subject	Year	Role
Thorlakson Foundation	Treatment of osteomyelitis of the foot in diabetic patients	1987	Co-PI
CanFAR	Rapid antiviral susceptibility testing	1991	PI
Banting Research Foundation	PCR-based antiviral susceptibility testing	1991	PI
OGH Research Foundation	Applications of PCR in HIV research	1991	PI
CanFAR	Antiviral effect of low-dose doxorubicin	1991	PI
CHEO Research Institute	Cross-sectional study of ZDV resistance among HIV-infected Canadian children	1992	Co-PI

CanFAR	Resistance of clinical isolates to ZDV, transmissibility and natural history	1992	PI
Musculoskeletal Transplant Foundation	Effects of gamma irradiation on HIV	1992	PI
Physicians Services Foundation	Rapid PCR-based antiviral susceptibility testing	1992-1993	PI
OGH Research Foundation	Rapid HIV antiviral susceptibility testing	1992	PI
Health & Welfare Canada	Evaluation of PCR as a diagnostic tool for HIV	1992	PI
Bickell Foundation	Transmission of HIV by bone transplantation	1993	PI
CanFAR	DNA amplification to monitor CMV disease in HIV infected patients	1993	PI
CanFAR	Immunoregulatory cytokines in HIV pathogenesis	1993	Co-PI
Sick Children Research Foundation	Cross-sectional study of ZDV resistance among HIV-infected Canadian children	1993-94	Co-PI
CanFAR	Viral load in genital secretions in HIV-infected women	1994	Co-PI
BCHRF	Application of clinical retrovirology to patient management	1995	PI
CanFAR	Effects of HIV on B7 expression by macrophages	1996	PI
ALDA Pharma NSERC	Evaluation of the antiretroviral activity of novel disinfectant preparations	1997	PI
CanFAR	Can antiretroviral therapy correct B cell abnormalities in HIV seropositive patients	1998	Co-PI
National Institutes of Health (NIH)	An integrated approach to primary HIV infection	1999-2003	PI
International Antiviral Therapy Evaluation Centre (IATEC)	Protocol 2NN: An open label comparative study to evaluate the antiviral efficacy and safety of Nevirapine and Efavirenz or both drugs combined in combination with d4T 3TC	1999-2003	PI
Medical Research Council (UK)	Open randomized trial to evaluate different therapeutic strategies of combination therapy for HIV-1 infection: the INITIO trial	2000-2006	PI
Canadian Institutes of Health Research	CTN 164: A Prospective Randomized Trial of Structured Treatment Interruption (STI) Followed by the Initiation of a New Antiretroviral Regimen Versus Immediate switching to a New Antiretroviral Regimen in the HIV-Infected Patients Experiencing Virologic Failure on HAART 2001	2001-2004	PI
Medical Research Council (UK)	CTN167: A Randomized, Clinical Trial of Novel Therapeutic Strategies for Patients in Whom Antiretroviral Therapy has failed	2001-2006	PI

Highbury Foundation	Immune Therapy of Acute/Early HIV Infection	2003	PI
Canadian HIV Trials Network	CTN147: A Pilot Study Assessing the Efficacy of Pneumococcal Vaccine in HIV Patients: Delayed versus Immediate Immunization	2003-2004	PI
Canadian HIV Trials Network	CTN157: A Randomized, Open-Label, Multicentre Trial Comparing the Effects of Fenofibrate and the Combination of Fenofibrate with L-Carnitine on Antiretroviral-Related Hypertriglyceridemia	2003-2004	PI
NIH/CPCRA	SMART Study: Strategic Management of Antiretroviral Therapy	2003-2008	PI
National Institutes of Health (NIH)	The use of time-limited HAART in the treatment of acute/early HIV infection	2004-2009	Co-PI
Vancouver Coastal Health	DOT Programs for the Treatment of HCV Infection in Inner City IDUs	2004	PI
Canadian Institutes of Health Research	Evaluation of a Multi-Disciplinary Approach for the Treatment of Hepatitis C in Injection Drug Users	2005-2009	PI
National Institutes of Health (NIH)	TL-HAART: A Randomized Trial of HAART in Acute/Early HIV Infection.	2005-2009	PI
Canadian Institutes of Health Research	Development of New Models for the Delivery of Antiretroviral Therapy to Injection Drug Users on the Downtown East side of Vancouver	2005-2007	Co-PI
Canadian HIV Trials Network/Canadian Institutes of Health Research	A Tri-National, Randomized, Controlled Trial to Determine the Optimal Management of Patients with HIV Infection for Whom First and Second-line Highly Active Antiretroviral Therapy has Failed.	2005-2007	PI
Canadian Institutes of Health Research	Effectiveness of Pegylated Interferon Plus Ribavirin in the Treatment of Active and Past Intravenous Drug Users Infected with Hepatitis C Virus	2006-2007	Co-PI
Canadian Institutes of Health Research	If Hepatitis C Virus (HCV) is an Opportunistic Infection, Why has HAART not led to Dramatic Improvements in Liver Disease Among HIV-HCV co-infected Persons?	2006-2009	Co-PI
Canadian HIV Trials Network	CTN 194: A Randomized, Placebo Controlled Trial of Cetalopram for the Prevention of Depression and its Consequences in HIV-Hepatitis C Co-infected Individuals Initiating Pegylated Interferon/Ribavirin Therapy.	2006-2009	PI
Canadian HIV Trials Network	CTN222: Canadian Co-infection Cohort Study	2007-	PI

Canadian HIV Trials Network	CTN 237: A controlled trial to assess the immunogenicity and efficacy of three influenza vaccine dosing strategies in HIV infected adults	2008-2009	PI
Women's College Hospital	PK in Women Project: Predictors of antiretroviral pharmacokinetics in HIV-infected women with virologic suppression on combination antiretroviral therapy	2008-2009	PI
Canadian HIV Trials Network	CTN 238: A randomized controlled clinical trial of micronutrient and antioxidant supplementation in persons with untreated HIV infection (MAINTAIN Study)	2009-2013	PI
Canadian HIV Trials Network	CTN 240: Valacyclovir in delaying antiretroviral treatment entry (VALIDATE Study)	2009-2013	PI
Canadian HIV Trials Network	CTN 247: The Canadian Cohort of HIV+ Slow Progressors: A Study of Host and Viral Factors Associated with Disease Progression in Long Term HIV Infected Subjects	2009-2014	PI
Canadian HIV Trials Network	CTN 260: A Randomized Prospective Open Label Study of Switching to Raltegravir Based ART Compared to Maintaining Ritonavir Boosted PI-based ART on Liver Fibrosis Progression in HIV-HCV Coinfected Patients	2012-2014	PI
Canadian HIV Trials Network	CTNPT014: An observational study of Kaletra®/Celsentri® combination therapy for the management of HIV infection in the setting of HCV co-infection	2012-2013	PI
Canadian HIV Trials Network	CTN 254: Predictive value of inflammatory biomarkers in untreated HIV disease progression, and their response to initiation of antiretroviral therapy	2012-2013	PI
Kirby Institute	ACTIVATE: A Phase IV, Open-label, Multicentre, International Trial of Response Guided Treatment With Directly Observed Pegylated Interferon Alfa 2b and Self Administered Ribavirin for Patients With Chronic HCV Genotype 2 or 3 and Ongoing Injection Drug Use	2012-2014	PI
Canadian Institutes of Health Research	CTN 264: Prospective Investigation of the Relationship Between Food Insecurity and Health and Behavioural Outcomes in HIV-HCV Co-Infections: Clues for Prevention Intervention	2013-2016	PI
Canadian Institutes of Health Research	CTN 272: The Canadian HIV and Aging Cohort – Determinants of Increased Risk	2014-2019	PI

	of Cardiovascular Diseases in Individuals Living With HIV		
Canadian HIV Trials Network	CTN 286: A Phase IV, Multicentre Randomized Prospective Open Label Study to Evaluate Whether Switching From Current cART to Triumeq in Addition to Adherence Support Will Enhance Virologic Control and Adherence in Vulnerable Populations Relative to Adherence Support Alone (TRIIADD)	2015-2017	PI
Merck Sharp & Dohme Corp.	Engaging High Risk Populations of Downtown Vancouver and Williams Lake through Hepatitis C and HIV Community Portable Pop-up Clinics	2016-2019	PI
Merck Sharp & Dohme Corp.	HIV Total Patient Care at VIDC	2016-2017	PI
Merck Sharp & Dohme Corp.	Engaging High Risk Populations of Downtown Vancouver and William Lake through Hepatitis C and HIV Community Portable Pop-up Clinics	2016-2017	PI
National Institute of Allergy and Infectious Diseases (NIAID)	A5332: Randomized Trial to Prevent Vascular Events in HIV (REPRIEVE)	2016-2017	PI
Kirby Institute	VHCRP1405: A Phase IV Open-label, Multicentre, International Trial of Paritaprevir/Ritonavir, Ombitasvir, Dasabuvir ±Ribavirin for Chronic Hepatitis C Virus Genotype 1 Infection and Recent Injection Drug Use or Receiving Opioid Substitution Therapy (D3FEAT)	2016-2017	PI
Kirby Institute	VHCRP1309: A Phase II, Open-label, Single Arm, Multicentre, International Trial of Sofosbuvir (SOF) and GS-5816 for People With Chronic Hepatitis C Virus Infection and Recent Injection Drug Use (SIMPLIFY)	2016-2018	PI
Canadian HIV Trials Network	CTN 283: The I-Score Study: The development and validation of a patient-reported measure of antiretroviral therapy's interference with life: a clinical patient-management tool for healthcare providers	2016-2017	PI
ViiV Healthcare	HIV Prevention & Sexual Health Promotion: Community – Based Workshops (PIHVOT)	2016-2018	PI
CIHR	Optimization of HCV and HIV care for vulnerable populations in Vancouver – A framework for long-term multidisciplinary care	2016-2018	PI

University of Calgary	Canadian HBV Network	2016-	Co-PI
CanHepC	The Canadian Network on Hepatitis C Clinical Research Core: National HCV Registry Protocol	2017-	Co-PI
CanHepC	The Canadian Network Undertaking against Hepatitis C (CANUHC) - A Longitudinal, Observational Study	2017-	PI
Canadian HIV Trials Network	CTN 299: Bone health in ageing women: Improvement or prevention of changes in Bone Mineral Density by Switching Antiretroviral Agents. Is there an optimal time to intervene? *(BEING)*	2017-	PI
Undisclosed	Safety and efficacy of B/F/TAF STR in HIV-infected patients with episodic transient viremia on multi-tablet regimens	2018-	PI
The Kirby Institute/ University of New South Wales Sydney	Direct-acting antiviral therapy and reinfection among people with chronic hepatitis C virus infection and recent injecting drug use recruited from community- based settings (SHARP-C)	2019-	PI
Canadian Institutes of Health Research (CIHR) and the Canadian HIV Trials Network	Evaluation of a tailored virtual intervention as an instrument of prevention and therapeutic support to improve the health of PLHIV and reduce comorbidity associated with HIV (LHIVE)	2019-	PI
Undisclosed	Micro-elimination of HCV infection among People Who Use Drugs (PWUD) in British Columbia: A comprehensive, multidisciplinary, scalable programmatic approach (CHIME)	2019-	PI
Undisclosed	Grand Plan: Efficacy of Glecaprevir/Pibrentasvir (G/P) for HCV-infected people who use drugs (PWUD) disengaged from health care: A systematic approach to engagement and HCV treatment	2019-	PI
None	Find 50 – Coordinating with Opioid Substitution Therapy (OST) prescribing physicians to identify and engage HCV-positive People Who Use Drugs (PWUD) in Surrey, British Columbia	2018-2019	PI
ViiV Healthcare	Engagement of HIV+ individuals in the inner-city populations: Towards achievement of the 90-90-90 goal in all infected sub-populations	2020	PI
Bill and Melinda Gates Foundation (Therapeutic Accelerator); Montreal Heart Institute	Colchicine Coronavirus SAR-CoV2 Trial (COLCORONA)	2020-	Co -PI

(A2) Clinical study involvement – Pharmaceutical sponsors

Granting Agency	Subject	Year	Role
Burroughs Wellcome	Randomized study of combination antiretroviral therapy with ZDV, ddI and Wellferon, virologic evaluations	1992	PV(1)
Burroughs Wellcome	p24 assays for the evaluation of the efficacy of ZDV therapy	1993	PI
Allellix Biopharmaceuticals	Evaluation of the antiretroviral activity of ALX40-4C in vitro	1993-1994	PI
Efamol Research	Evaluation of the antiretroviral activity and toxicity of lithium gamma linolenic acid, LiGLA	1993-1999	PI
Chiron	Evaluation of bDNA to measure viral load	1994	PI
Boehringer Ingelheim	Randomized placebo-controlled study of ZDV, ddI and nevirapine combinations in drug-naïve HIV-infected patients, virologic studies	1994-1996	PV(1)
Roche	Trial of ZDV, ddC and saquinavir combinations in drug- naïve HIV-infected patients, clinical and virological studies	1994-1995	PI
Pharmacia & Upjohn	Randomized placebo-controlled study of ZDV, 3TC, ddI and delavirdine combinations in drug-naïve and experienced HIV-infected patients	1994-1996	PI
Receptagen	Evaluation of the antiretroviral activity of CoQ10	1995	PI
Roche Diagnostics	Evaluation of the Amplicor to measure viral load	1995	PI
Bristol Myers Squibb	Evaluation of combination therapy with ddI and Hydroxyurea	1995	PV(1)
Roche	Evaluation of the efficacy of a new formulation of saquinavir	1995-1998	PI
Efamol Research	Evaluation of the efficacy of LiGLA in vivo, a phase I study	1996	Co-PI
Bristol Myers Squibb	A pilot, open label, compassionate study of the antiretroviral effect of combination therapy with d4T and 3TC among HIV-infected patients intolerant to ZDV therapy	1996	PV(1)
Merck Sharp Dome	Evaluation of combination therapy with nevirapine, indinavir and 3TC	1996	PV(1)
Roche	Evaluation of combination therapy with saquinavir, ritonavir and nevirapine in post-primary HIV infection	1997	PI
Pharmacia & Upjohn	Evaluation of combinations of ZDV, d4T, 3TC, delavirdine and saquinavir (new formulation) in drug-I HIV-infected individuals, clinical and virologic studies	1998	PI & PV(1)
Abbott Laboratories	A randomized, open-label, Phase III study of ABT/ritonavir in Combination with Nevirapine and Two Nucleoside Reverse Transcriptase Inhibitors vs. investigator selected protease inhibitors in combination with nevirapine and two NRTIs in antiretroviral-experienced HIV-infected subjects	2000-2002	PI
Bristol-Myers Squibb	A Phase III study comparing the antiviral efficacy and safety of BMS-232632 with Efavirenz; each in combination with fixed dose Zidovudine-Lamivudine, Protocol 1424-034	2001	PI

Agouron Pharmaceuticals	Phase IV evaluation of delavirdine in salvage therapy (DELSA)	2001-2002	PI
Schering	A PhaseIV Open-Label Study of Patient Outcomes and the factors associated with patient compliance and outcome with Rebetron therapy in patients with chronic Hepatitis C who are stabilized in a Methadone Maintenance Treatment Program	2001-2003	PI
Triangle Pharmaceuticals	A randomized, double-blind, equivalence trial comparing Emtricitabine to Stavudine within a triple drug combination containing Didanosine plus Efavirenz in Antiretroviral-drug naïve HIV-1 infected patients	2001-2003	PI
Triangle Pharmaceuticals	A Phase I/II Study to Evaluate the Safety, Tolerability, Pharmacokinetics and Antiviral Activity of L-FMAU after escalating Oral Doses in Patients Infected with Hepatitis B Virus	2001	PI
Schering	Treatment of HCV-Infected IVDUs: Observational Studies	2001-2005	PI
Boehringer Ingelheim Foundation	Use of antiretroviral therapy in intravenous drug users (IVDUs) – Novel Models of Care	2002-2005	PI
Abbott Laboratories	QD Study: A Randomized Controlled Trial of once-daily Tenofovir, 3TC and lopinavir/r versus remaining on the same regimen in HIV-infected patients virologically suppressed on their first PI-containing HAART Regimen	2003-2004	PI
Abbott Laboratories	Directly Observed Therapy (DOT) for the Management of Intravenous Drug Users (IVDUs) Enrolled in a Methadone (MET) Program A Pilot Study of Tenofovir and Kaletra®	2003-2004	PI
Agouron Pharmaceuticals	Nelfinavir (NFV)-based HAART for the treatment of intravenous drug users (IDUs) enrolled in a methadone (MET) treatment program	2003	PI
Boehringer Ingelheim	RESIST Program: Phase III studies to evaluate the efficacy of Tipranavir in patients with HIV resistant to multiple protease inhibitors	2003-2004	PI
Pfizer Pharmaceuticals	Administration of Azithromycin under maximally assisted therapy (MAT) for the Treatment of Respiratory and Skin and Soft Tissue Infections in Intravenous Drug Users (IVDUs) enrolled in a Methadone maintenance program in Vancouver	2003	PI
Pharmacia & Upjohn	Administration of Linezolid under maximally assisted therapy (MAT) for the Treatment of Skin and Soft Tissue Infections in Intravenous Drug Users (IVDUs) enrolled in a Methadone maintenance program in Vancouver	2003-2006	PI
Bristol Myers Squibb	Evaluation of Atazanavir Substitution Intervention (EASI)	2004-2006	PI
Pfizer Pharmaceuticals	Nelfinavir Substitution to Enhance Adherence: An Observational Study	2004-2006	PI

Boehringer Ingelheim	Long-Term Strategies for The Treatment of HIV-Infected IDUs	2004-2006	PI
Boehringer Ingelheim	Tipranavir 1182.33: A Randomized, Open Label, Active Controlled Trial to Evaluate the Antiviral Efficacy and Safety of Treatment with 500mg Tipranavir Plus 100mg or 200mg Ritonavir p.o. BID in Combination with Standard Background Regimen in Comparison to 400mg Lopinavir Plus 100mg Ritonavir p.o. BID in Combination with Standard Background Regimen in Antiretroviral Therapy Naïve Patients for 48 weeks with Extension up to 156 weeks	2004-2007	PI
GlaxoSmithKline	HPR20001: A Phase IIB Randomized, Multicenter, Parallel Group Study to Evaluate the Short-Term Safety, Pharmacokinetics and Antiviral Activity of Four Blinded Dosing Regimens of GW640385/Ritonavir Therapy Compared to Open-label Current Protease Inhibitor Therapy in HIV-1-Infected, Protease Inhibitor-Experienced Adults for 2 weeks with Long-Term Evaluation of Safety, Pharmacokinetics and Antiviral Activity of Selected GW640385/Ritonavir-boosted, Protease Inhibitor Containing Regimens	2005-2007	PI
GlaxoSmithKline	APV102002: A Phase III, Randomized, Controlled, Open-label, Multicenter, Three Arm Study to Compare the Efficacy and Safety of a Dual-boosted HIV-1 Protease Inhibitor regimen of Fosamprenavir (FPV)/Lopinavir (LPV)/Ritonavir (RTV) 1400mg/533mg/133mg Twice Daily (BID) and Increased Dosage Regimen of FPV/RTV 700mg/100mg BID for 24 weeks in Multiple-PI Experienced, HIV-infected Adults Experiencing Virological Failure	2005-2007	PI
Pfizer Pharmaceuticals	A4001026: A Multicenter, Randomized, Double-Blind, Comparative Trial of A Novel CCR5 Antagonist, In Combination with Zidovudine/Lamivudine Versus Efavirenz in Combination With Zidovudine Lalamivudine for the Treatment of Antiretroviral-naïve HIV-1 Infected Subjects	2005-2007	PI
Pfizer Pharmaceuticals	A4001027: A Multicenter, Randomized, Double-blind, Placebo-controlled Trial of A Novel CCR5 Antagonist, In Combination with Optimized Background Therapy Versus Optimized Background Therapy Alone for the Treatment of Antiretroviral-experienced HIV-1 Infected Subjects	2005-2007	PI
Hoffman La Roche Ltd.	Intense: A Phase IIIb/IV Randomized, Controlled Study Evaluating and Intensification Treatment Strategy of Adding Enfuvirtide to an Oral Highly Active Antiretroviral Therapy (HAART) in Treatment Experienced Patients	2006-2008	Co-PI

Gilead Sciences	TEN Switch: An Observational Phase IV Study to Evaluate the Efficacy of Substituting Tenofovir for Didanosine in HIV-infected Subjects responding to HAART and requiring therapy for hepatitis C virus infection.	2006-2007	PI
Conjuchem Inc.	A Multicenter, Placebo-controlled, Double-blind, Phase 2 Study to Evaluate the Efficacy and Safety of CJC-1295 Administered for 12 weeks in HIV-infected Subjects with HIV-associated Visceral Obesity	2006-2007	PI
Boehringer Ingelheim	A Long-term Open-label, Rollover Trial Assessing the Safety and Tolerability of Combination Tipranavir and Ritonavir Use in HIV-1 Infected Subjects	2006-2007	PI
Abbott Laboratories	M05-730: A Phase 3, Randomized, Open-label Study of Lopinavir/ritonavir Tables Versus Soft Gel Capsules and Once Daily Versus Twice Daily Administration, When Co-administered with NRTI's in Antiretroviral Naïve HIV-Infected Subjects.	2006-2008	PI
Novartis Pharmaceuticals	CFAM810A2308: A multicenter, randomized, double-blind study to compare the efficacy and safety of patient-initiated famciclovir 1000 mg b.i.d. x 1 day to valacyclovir 500 mg b.i.d. x 3 days in immunocompetent adults with recurrent genital herpes.	2006-2008	PI
Avexa Limited	AVX-301: A phase 2b/3, randomized, double blind, dose confirming study of the safety, efficacy and tolerability of apricitabine versus lamivudine in treatment-experienced HIV-1 infected patients with the M184V/I mutation in reverse transcriptase.	2008-2010	PI
Avexa Limited	AVX-303: A phase 3, open-label 96-week extension study of the safety of apricitabine in the treatment-experienced HIV-1 infected patients who have completed protocol AVX-301 or AVX-302 or who have met the criteria for open-label access to ATC because of virologic failure/lack of response.	2008-2011	PI
Boehringer Ingelheim	BI 1100.1486: A randomized, double-blind, double dummy, parallel group, active controlled trial to evaluate the antiviral efficacy of 400 mg QD neVirapine Extended Release formulation in comparison to 200m BID neVirapine immediate release in combination with Truvada in antiretroviral therapy naïve HIV-1 infected subjects.	2008-2010	PI
Pharmasset, Inc.	CI-PSI-5268-06-305: A multi-center, randomized, double-blind, active-control, 96 week, phase 3 trial of the efficacy and safety of clevudine compared with adefovir at weeks 48 and 96 in nucleoside treatment-naïve patients with HBeAg positive chronic hepatitis due to Hepatitis b Virus.	2008-2011	PI
Pharmasset, Inc.	CI-PSI-5268-06-306: A multi-center, randomized, double-blind, active-control, 96 week, phase 3 trial of the efficacy and safety of clevudine compared with adefovir at weeks 48 and 96 in nucleoside treatment-	2008-2011	PI

	naïve patients with HBeAg negative chronic hepatitis B Virus.		
Abbott Laboratories	A06-321: A pilot, prospective, open-label study to evaluate the safety and efficacy of Kaletra monotherapy in HIV/HCV co-infected subjects.	2008-2010	PI
Gilead Sciences	GS-US-183-0145: A Multicenter, Randomized, Double-Blind, Double-Dummy, Phase 3 Study of the Safety and Efficacy of Ritonavir-Boosted Elvitegravir (EVG/r) Versus Raltegravir (RAL) Each Administered With a Background Regimen in HIV-1 Infected, Antiretroviral Treatment-Experienced Adults	2008-2013	PI
Pfizer Pharmaceuticals	Pfizer A4001067: An International, Multicenter, Prospective Observational Study of the Safety of Maraviroc used with Optimized Background Therapy in Treatment-Experienced HIV-1 Infected Patients (POEM)	2008-2013	PI
Merck Sharp & Dohme Corp.	Pegetron® Redipen™ Prospective Optimal Weight-Based Dosing Response Program (P04423)	2009-2012	PI
Boehringer Ingelheim	BI 1220.40: Antiviral effect and safety of once daily BI 201335 NA in hepatitis C virus genotype 1 infected treatment-naïve patients for 12 or 24 weeks as combination therapy with pegylated interferon-α 2a and ribavirin (open label, randomised, Phase II).	2009-2011	PI
Boehringer Ingelheim	BI 1220.5: Antiviral effect, safety and pharmacokinetics of once daily BI 201335 NA in hepatitis C virus genotype 1 infected treatment-naïve patients for 24 weeks as combination therapy with pegylated interferon-α 2a and ribavirin (double blinded, randomised, placebo controlled, Phase II)	2009-2011	PI
Pfizer Pharmaceuticals	A8121014: A Phase 2, Randomized, Double - Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of Filibuvir Plus Pegylated Interferon Alfa-2 and Ribavirin in Treatment Naive, HCV Genotype 1 Infected Subjects (FITNESS)	2009-2011	PI
Vertex Pharmaceuticals	VCH222-102: A Phase Ib/IIa, Multicenter, Randomized, Double-Blinded, Placebo-Controlled Study of the Antiviral Activity, Safety, Tolerability, and Pharmacokinetics of Multiple Ascending Doses of VCH-222 in Subjects with Chronic Hepatitis C-Infection	2009-2012	PI
Gilead Sciences	GS-US-196-0123: A Phase 2b, Randomized, Double-Blind, Placebo-Controlled Trial Evaluating 16 and 24 Weeks of Response Guided Therapy with GS-9190, GS-9256, Ribavirin (Copegus®) and Peginterferon Alfa 2a (Pegasys®) in Treatment Naïve Subjects with Chronic Genotype 1 Hepatitis C Virus Infection	2010-2013	PI
GlaxoSmithKline	ING114467: A Phase 3, randomized, double-blind study of the safety and efficacy of GSK1349572 plus abacavir/lamivudine fixed-dose combination therapy	2010-2013	PI

	administered once daily compared to Atripla over 96 weeks in HIV-1 infected antiretroviral therapy naive adult subjects (SINGLE)		
Anadys Pharmaceuticals	ANA598-505: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Trial of the Safety and Efficacy of ANA598 Administered with Pegylated Interferon and Ribavirin in Genotype 1 Patients with Chronic Hepatitis C Infection	2011-2013	PI
Boehringer Ingelheim	BI 1220.47: A phase III, randomised, double-blind and placebo-controlled study of once daily BI 201335 120 mg for 12 or 24 weeks or BI 201335 240 mg for 12 weeks in combination with pegylated interferon-α and ribavirin in treatment-naïve patients with genotype 1 chronic hepatitis C infection	2011-2013	PI
Gilead Sciences	GS-US-264-0110: A Phase 3B, Randomized, Open-label Study to Evaluate the Safety and Efficacy of a Single Tablet Regimen of Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate Compared with a Single Tablet Regimen of Efavirenz/Emtricitabine/Tenofovir Disoproxil Fumarate in HIV 1 Infected, Antiretroviral Treatment-Naïve Adults	2011-2013	PI
Gilead Sciences	GS-US-264-0106: A Phase 3 Randomized, Open Label Study to Evaluate Switching from Regimens Consisting of a Ritonavir-boosted Protease Inhibitor and Two Nucleoside Reverse Transcriptase Inhibitors to Emtricitabine/Rilpivirine/Tenofovir Disoproxil Fumarate (FTC/RPV/TDF) Fixed-dose Regimen in Virologically Suppressed, HIV 1 Infected Patients	2011-2012	PI
Boehringer Ingelheim	BI1220.7: A phase III, randomised, double-blind and placebo controlled study of once daily BI 201335, 240 mg for 12 or 24 weeks in combination with pegylated interferon-α and ribavirin in patients with genotype 1 chronic hepatitis C infection who failed a prior PegIFN/RBV treatment	2011-2013	PI
Boehringer Ingelheim	BI1220.48: A phase III, open-label study of once daily BI 201335 240 mg for 24 weeks in combination with pegylated interferon-α (PegIFN) and ribavirin (RBV) in patients with genotype 1 chronic hepatitis C infection who failed a prior PegIFN / RBV treatment	2011-2013	PI
Gilead Sciences	GS-US-248-0132: A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study of GS-5885, GS-9451, Tegobuvir and Ribavirin; GS-5885, GS-9451 and Tegobuvir; GS-5885, GS-9451 and Ribavirin in Interferon Ineligible or Intolerant Subjects with Chronic Genotype 1a or 1b HCV Infection	2011-2012	PI
GlaxoSmithKline	ING112574: A Phase III study to demonstrate the antiviral activity and safety of dolutegravir in HIV-1 infected adult subjects with treatment failure on an integrase inhibitor containing regimen.	2011-2013	PI

GlaxoSmithKline	ING111762: A Randomized, Double-blind Study of the Safety and Efficacy of GSK1349572 50 mg Once Daily Versus Raltegravir 400 mg Twice Daily, Both Administered with an Investigator-selected Background Regimen Over 48 Weeks in HIV-1 Infected, Integrase Inhibitor-Naïve, Antiretroviral-Experienced Adults	2011-2012	PI
Bristol-Myers Squibb	AI444-043: A Phase 3, Open Label Study of Safety and Efficacy With BMS-790052 Plus Peg-Interferon Alfa 2a and Ribavirin in Previously Untreated HCV Patients Coinfected With Human Immunodeficiency Virus (HIV) and Hepatitis C Virus (HCV)	2011-2013	PI
Boehringer Ingelheim	BI1241.27: A Multi-centre, Open Label, Parallel Group Trial to Evaluate the Pharmacokinetic Interactions Between BI 207127 (600 mg t.i.d. or 600 mg b.i.d.) and BI 201335 (120 mg q.d.) Given in Combination With Ribavirin for 24 Weeks, and Their Combined Effect on the Pharmacokinetics of Tenofovir, Raltegravir, Caffeine (the Probe Drug Substrate for CYP1A2), Tolbutamide (the Probe Drug Substrate for CYP2C9) and Midazolam (the Probe Drug Substrate for CYP3A4) in Treatment naïve Patients and Prior Treatment Relapse or Partial Responder Patients With Genotype 1 Chronic Hepatitis C Infection	2012-2014	PI
Gilead Sciences	GS-UX-174-0172: Prospective, Observational, Post-Marketing Renal Safety Surveillance Registry in Patients with Chronic Hepatitis B (HBV) Infection with Decompensated Liver Disease Receiving Nucleotide/side Therapy on the Orthotopic Liver Transplant (OLT) List	2012-2014	PI
GlaxoSmithKline	LAI116482: A Phase IIb, dose ranging study of oral GSK1265744 in combination with nucleoside reverse transcriptase inhibitors for induction of HIV-1 virologic suppression followed by an evaluation of maintenance of virologic suppression when oral GSK1265744 is combined with oral rilpivirine in HIV-1 infected, antiretroviral therapy naïve adult subjects	2012-2014	PI
Bristol-Myers Squibb	AI467003: A Phase IIb Randomized, Controlled, Partially Blinded Clinical Trial to Investigate Safety, Efficacy and Dose-response of BMS-986001 in Treatment-naive HIV-1-infected Subjects, Followed by an Open-label Period on the Recommended Dose	2012-2013	PI
Cubist Pharmaceuticals	LCD-CDAD-10-07: A Randomized, Double-Blinded, Active-Controlled Study of CB-183,315 in Patients With Clostridium Difficile Associated Diarrhea	2012-2014	PI
Merck Sharp & Dohme Corp.	A Prospective Observational **S**tudy **I**nvestigating the **M**anagement of G1 Chronic Hepatitis C Adult **P**atients Treated with VICTRELIS™ (boceprevir) in Combination with Peginterferon Alpha / Ribavirin: A Real **L**if**E** Trial in Canada (**S.I.M.P.L.E.** – Canada)" having protocol number MK-3034-116-00,	2012-2014	PI

Gilead Sciences	GS-US-248-0122: A Long Term Follow-up Registry for Subjects Who Achieve a Sustained Virologic Response to Treatment in Gilead-Sponsored Trials in Subjects With Chronic Hepatitis C Infection	2012-2014	PI
Gilead Sciences	GS-US-248-0123: A Long Term Follow-up Registry for Subjects Who Achieve a Sustained Virologic Response to Treatment in Gilead-Sponsored Trials in Subjects With Chronic Hepatitis C Infection	2012-2014	PI
Gilead Sciences	GS-US-334-0108: A Phase 3, Multicenter, Randomized, Double-Blind, Study to Investigate the Efficacy and Safety of GS-7977 + Ribavirin for 12 or 16 Weeks in Treatment Experienced Subjects With Chronic Genotype 2 or 3 HCV Infection	2012-2013	PI
Pfizer Pharmaceuticals	A4001095: A Multicenter, Randomized, Double Blind, Comparative Trial Of Maraviroc + Darunavir/Ritonavir Versus Emtricitabine/Tenofovir +Darunavir/Ritonavir For The Treatment Of Antiretroviral-Naïve HIV Infected Patients With Ccr5 Tropic HIV 1	2012-2014	PI
Roche	NP27946: A Study to Evaluate Safety, Tolerability, Pharmacokinetics and Antiviral Activity of Ritonavir-Boosted DANOPREVIR and RO5024048 in Different Combinations in Null Responder or Treatment Naïve Patients With Chronic Hepatitis C and Compensated Cirrhosis	2012-2013	PI
Janssen Inc.	POISE: PREZISTA or INTELENCE Switch Evaluation in Virologically Suppressed Patients Naïve to Darunavir or Etravirine and Who Are Intolerant of Their Current or Prior Combination Antiretroviral Therapy Regimen: A Phase IV, Open-label, Multicentre Observational Trial	2012-2013	PI
Bristol-Myers Squibb	AI444-046: A Long-Term Follow-up Study of Subjects Who Participated in a Clinical Trial in Which Asunaprevir (BMS-650032) and/or Daclatasvir (BMS-790052) Was Administered for the Treatment of Chronic Hepatitis C	2013-2014	PI
Bristol-Myers Squibb	AI452-032: Phase 3 Open Label Study Evaluating the Efficacy and Safety of Pegylated Interferon Lambda-1a, in Combination With Ribavirin and Daclatasvir, for Treatment of Chronic HCV Infection With Treatment naïve Genotypes 1, 2, 3 or 4 in Subjects Co-infected With HIV	2013-2014	PI
Boehringer Ingelheim	BI1241.20: A Phase III, Randomized, Partially Double-Blind and Placebo-Controlled Study of BI 207127 in Combination With Faldaprevir and Ribavirin in Treatment-Naive Patients With Chronic Genotype 1 HCV Infection	2013-2014	PI
Gilead Sciences	GS-US-334-0109: An Open-Label Study of GS-7977 + Ribavirin With or Without Peginterferon Alfa-2a in Subjects With Chronic HCV Infection Who Participated in Prior Gilead HCV Studies	2013-2014	PI

Gilead Sciences	GS-US-292-0109: A Phase 3, Open-Label Study to Evaluate Switching From a TDF-containing Combination Regimens to a TAF-Containing Combination Single Tablet Regimen (STR) in Virologically-suppressed, HIV-1 Positive Subjects	2013-2014	PI
GlaxoSmithKline	200056-A Phase IIb Study Evaluating a Long-Acting Intramuscular Regimen of GSK1265744 plus TMC278 For The Maintenance of Virologic Suppression Following an Induction of Virologic Suppression on an Oral Regimen of GSK1265744 Plus Abacavir/Lamivudine in HIV-1 Infected, Antiretroviral Therapy-Naïve Adult Subjects	2013-2017	PI
ViiV Healthcare	201147: A Phase IIIb, randomized, open-label study of the safety, efficacy, and tolerability of switching to a fixed dose combination of abacavir/dolutegravir/lamivudine from current antiretroviral regimen in HIV-1 infected adults who are virologically suppressed	2013-2015	PI
Bristol-Myers Squibb	AI443-102: A Phase 3 evaluation of a daclatasvir/asunaprevir/BMS-791325 fixed dose combination in non-cirrhotic subjects with genotype 1 chronic hepatitis	2013-2014	PI
Bristol-Myers Squibb	AI443-113: A Phase 3 evaluation of a daclatasvir/asunaprevir/BMS-791325 fixed dose combination in subjects with genotype 1 chronic hepatitis C and compensated cirrhosis	2013-2014	PI
Gilead Sciences	GS-US-320-0108: A Phase 3, Randomised, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300mg QD for the Treatment of HBeAg-Positive, Chronic Hepatitis B	2013-2016	PI
Gilead Sciences	GS-US-320-0110: A Phase 3, Randomised, Double-Blind Study to Evaluate the Safety and Efficacy of Tenofovir Alafenamide (TAF) 25mg QD versus Tenofovir Disoproxil Fumarate (TDF) 300mg QD for the Treatment of HBeAg-Negative, Chronic Hepatitis B	2013-2016	PI
Gilead Sciences	GS-US-334-0153: A Phase 3B Randomized, Open-Label, Multi-Center Trial Assessing Sofosbuvir + Ribavirin for 16 or 24 Weeks and Sofosbuvir + Pegylated Interferon + Ribavirin for 12 Weeks in Subjects With Genotype 2 or 3 Chronic HCV Infection.	2013-2015	PI
AbbVie	M13-774: A Randomized, Open-Label Study to Evaluate the Efficacy and Safety of ABT-450/Ritonavir/ABT-267 and ABT-333 Co-administered With and Without Ribavirin Compared to Telaprevir Co-administered With Pegylated Interferon α-2a and Ribavirin in Treatment-Naïve Adults With Chronic Hepatitis C Genotype 1 Virus Infection (MALACHITE I)	2013-2015	PI

Merck Sharp & Dohme Corp.	MK1439-007: Multicenter, Double-Blind, Randomized, 2-Part, Dose Ranging Study to Compare the Safety, and Antiretroviral Activity of MK-1439 Plus TRUVADA Versus Efavirenz Plus TRUVADA in Antiretroviral Treatment-Naive, HIV-1 Infected Patients	2013-2015	PI
Merck Sharp & Dohme Corp.	MK5172-061: A Phase III Open-Label Clinical Trial to Study the Efficacy and Safety of the Combination Regimen Grazoprevir (GZR) and Elbasvir (EBR) in Treatment-Naïve Subjects With Chronic HCV GT1, GT4, and GT6 Infection Who Are Co-Infected With HIV	2014-2015	PI
Merck Sharp & Dohme Corp.	MK5172-062: A Phase III Open-Label Clinical Trial to Study the Efficacy and Safety of the Combination Regimen of MK-5172/MK-8742 in Treatment- Naïve Subjects with Chronic HCV GT1, GT4, GT5, and GT6 Infection who are on Opiate Substitution Therapy	2014-2015	PI
Merck Sharp & Dohme Corp.	MK0518-292: A Phase III Multicenter, Double-Blind, Randomised, Active Comparator-Controlled Clinical Trial to Evaluate the Safety and Efficacy of Reformulated Raltegravir 1200mg Once Daily Versus Raltegravir 400mg Twice Daily, Each in Combination with TRUVADA, in Treatment-Naïve HIV-1 Infected Subjects	2014-2015	PI
Bristol-Myers Squibb	AI452-016: A Long-Term Follow-Up Study of Subjects Who Participated in a Clinical Trial in Which Peginterferon Lambda-1a (BMS-914143) was Administered for the Treatment of Chronic Hepatitis C	2014-2016	PI
Gilead Sciences	GS-US-342-1138: A Phase 3, Multicenter, Randomised, Double-Blind, Placebo-Controlled Study to Investigate the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 Weeks in Subjects with Chronic HCV	2014-2015	PI
Gilead Sciences	GS-US-342-1140: A Phase 3, Multicenter, Randomised, Open-Label Study to compare the Efficacy and Safety of Sofosbuvir/GS-5816 Fixed Dose Combination for 12 Weeks with Sofosbuvir and Ribavirin for 24 Weeks in Subjects with Chronic Genotype 3 HCV Infection	2014-2015	PI
Gilead Sciences.	GS-US-311-1089: A Phase 3, Randomized, Double-Blind, Switch Study to Evaluate F/TAF in HIV-1 Positive Subjects who are Virologically Suppressed on Regimens Containing FTC/TDF	2014-2016	PI
Gilead Sciences	GS-US-292-0119: A Phase 3 Open-Label Study to Evaluate Switching from Optimized Stable Antiretroviral Regimens Containing Darunavir to Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide (E/C/F/TAF) Single Tablet Regimen (STR) plus Darunavir (DRV) in Treatment Experienced HIV-1 Positive Adults	2014-2015	PI

Gilead Sciences	GS-US-292-0117: A Phase 3, Two-Part Study to Evaluate the Efficacy of Tenofovir Alafenamide versus Placebo Added to a Failing Regimen Followed by Treatment with Elvitegravir/Cobicistat/Emtricitabine/Tenofovir Alafenamide in HIV-1 Positive, Antiretroviral Treatment-Experienced Adults	2014-2015	PI
F. Hoffman-La Roche	NV22688: A long-Term Monitoring Study To Evaluate The Presence Of Direct Acting Antiviral (DAA) Treatment-Resistant Mutations Or The Durability Of Sustained Virological Response (SVR) In Patients Treated With DAA-Containing Regimens for Chronic Hepatitis C Infection (CHC)	2014-2015	PI
Gilead Sciences	SINNR: STRIBILD in Non-Nucleoside Resistant Patients, An Evaluation of Safety and Efficacy in Vulnerable Populations	2014-2015	PI
Bristol-Myers Squibb	AI438047: A Multi-arm Phase 3 Randomized Placebo Controlled Double Blind Clinical Trial to Investigate the Efficacy and Safety of BMS-663068 in Heavily Treatment Experienced Subjects Infected With Multi-drug Resistant HIV-1	2015-2016	PI
Merck Sharp & Dohme Corp.	MK-1439A-024: A Phase III Multicenter, Open-Label, Randomized Study to Evaluate a Switch to MK-1439A in HIV-1-Infected Subjects Virologically Suppressed on a Regimen of a Ritonavir-boosted Protease Inhibitor and Two Nucleoside Reverse Transcriptase Inhibitors (NRTIs)	2015-2020	PI
AbbVie	M15-684: An Open-Label, Single Arm Study to Evaluate the Safety and Efficacy of Ombitasvir/Paritaprevir/Ritonavir and Dasabuvir in Treatment-Naïve Adults With Genotype 1b Hepatitis C Virus (HCV) Without Cirrhosis (GARNET)	2015-2016	PI
AbbVie	P15-651: Real World Evidence of the Effectiveness of Paritaprevir/r - Ombitasvir, ± Dasabuvir, ± Ribavirin in Patients With Chronic Hepatitis C - An Observational Study in Canada (AMBER)	2015-2017	PI
AbbVie	M14-004: A Multipart, Open-label Study to Evaluate the Safety and Efficacy of Ombitasvir/Paritaprevir/Ritonavir With and Without Dasabuvir Coadministered With and Without Ribavirin in Adults With Genotype 1 or 4 Chronic Hepatitis C Virus Infection and Human Immunodeficiency Virus, Type 1 Coinfection (TURQUOISE-I)	2015-2016	PI
Merck Sharp & Dohme Corp.	MK-1439-018: A Phase 3 Multicenter, Double-Blind, Randomized, Active Comparator-Controlled Clinical Trial to Evaluate the Safety and Efficacy of Doravirine (MK-1439) 100 mg Once Daily Versus Darunavir 800 mg Once Daily Plus Ritonavir 100 mg Once Daily, Each in Combination With TRUVADA™ or EPZICOM™/KIVEXA™, in Treatment-Naïve HIV-1 Infected Subjects	2015-2018	PI

AbbVie	M14-423: An Open-Label, Multicenter Study to Evaluate Long-Term Outcomes With ABT-450/Ritonavir/ ABT-267 (ABT-450/r/ABT-267) and ABT-333 With or Without Ribavirin (RBV) in Adults With Genotype 1 Chronic Hepatitis C Virus (HCV) Infection (TOPAZ-I)	2015-2020	PI
Gilead Sciences	GS-US-337-1431: A Registry for Subjects With Cirrhosis Who Achieve a Sustained Virologic Response Following Treatment With a Sofosbuvir-Based Regimen Without Interferon for Chronic Hepatitis C Infection	2015-2019	PI
Merck Sharp & Dohme Corp.	MK-5172-017: A Registry for Subjects With Cirrhosis Who Achieve a Sustained Virologic Response Following Treatment With a Sofosbuvir-Based Regimen Without Interferon for Chronic Hepatitis C Infection	2015-2018	PI
ViiV Healthcare	201636: A Phase III, Randomized, Multicenter, Parallel-group, Non-inferiority Study Evaluating the Efficacy, Safety, and Tolerability of Switching to Dolutegravir Plus Rilpivirine From Current INI-, NNRTI-, or PI-based Antiretroviral Regimen in HIV-1-Infected Adults Who Are Virologically Suppressed (SWORD-1)	2015-2018	PI
Janssen R&D Ireland	TMC114IFD3013: A Phase 3, Randomized, Active-controlled, Open-label Study to Evaluate the Efficacy, Safety and Tolerability of Switching to a Darunavir/ Cobicistat/ Emtricitabine/ Tenofovir Alafenamide (D/C/F/TAF) Once-daily Single-tablet Regimen Versus Continuing the Current Regimen Consisting of a Boosted Protease Inhibitor (bPI) Combined With Emtricitabine/Tenofovir Disoproxil Fumarate (FTC/TDF) in Virologically-suppressed, Human Immunodeficiency Virus Type 1 (HIV-1) Infected Subjects (EMERALD)	2015-2017	PI
Merck Sharp & Dohme Corp.	CAPICA 0000-369	2015-2016	PI
Janssen Pharmaceuticals, Inc.	00012432: PREZCOBIX Switch Evaluation in Virologically Suppressed HIV-infected patients	2015-2017	PI
Gilead Sciences	GS-US-367-1171: A Phase 3, Global, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Investigate the Safety and Efficacy of Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination for 12 Weeks in Direct-Acting Antiviral-Experienced Subjects With Chronic HCV Infection (POLARIS-1)	2016-2017	PI
Gilead Sciences	GS-US-367-1172: A Phase 3, Global, Multicenter, Randomized, Open-Label Study to Investigate the Safety and Efficacy of Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination for 8 Weeks Compared to Sofosbuvir/Velpatasvir for 12 Weeks in	2016-2017	PI0153

	Direct-Acting Antiviral-Naïve Subjects With Chronic HCV Infection (POLARIS-2)		
Gilead Sciences	GS-US-367-1173: A Phase 3, Global, Multicenter, Randomized, Open-Label Study to Investigate the Safety and Efficacy of Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination for 8 Weeks and Sofosbuvir/Velpatasvir for 12 Weeks in Subjects With Chronic Genotype 3 HCV Infection and Cirrhosis (POLARIS-3)	2016-2017	PI
Gilead Sciences	GS-US-367-1170: A Phase 3, Global, Multicenter, Randomized, Open-Label Study to Investigate the Safety and Efficacy of Sofosbuvir/Velpatasvir/GS-9857 Fixed-Dose Combination for 12 Weeks and Sofosbuvir/Velpatasvir for 12 Weeks in Direct-Acting Antiviral-Experienced Subjects With Chronic HCV Infection Who Have Not Received an NS5A Inhibitor (POLARIS-4)	2016-2017	PI
Gilead Sciences	GS-US-311-1717: A Phase 3b, Randomized, Double-Blind, Switch Study to Evaluate F/TAF in HIV-1 Infected Subjects Who Are Virologically Suppressed on Regimens Containing ABC/3TC	2016-2018	PI
AbbVie	M14-172: A Single Arm, Open-label Study to Evaluate the Efficacy and Safety of ABT-493/ABT-530 in Adults With Chronic Hepatitis C Virus Genotype 1, 2, 4, 5 or 6 Infection and Compensated Cirrhosis (EXPEDITION-1)	2016-2017	P15PI
ViiV Healthcare	205543: A Phase III, Randomised, Double-blind, Multicentre, Parallel-group, Non-inferiority Study Evaluating the Efficacy, Safety, and Tolerability of Dolutegravir Plus Lamivudine Compared to Dolutegravir Plus Tenofovir/Emtricitabine in HIV-1-infected Treatment-naïve Adults (GEMINI 2)	2016-2020	PI
ViiV Healthcare	201584: A Phase III, Randomized, Multicenter, Parallel-group, Open-Label Study Evaluating the Efficacy, Safety, and Tolerability of Long-Acting Intramuscular Cabotegravir and Rilpivirine for Maintenance of Virologic Suppression Following Switch from an Integrase Inhibitor Single Tablet Regimen in HIV-1 Infected Antiretroviral Therapy Naive Adult Participants (FLAIR)	2016-2021	PI
SHIRE	SHP 626-201: "A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)"	2016-2017	PI
Merck Sharp & Dohme Corp.	An Epidemiological Retrospective Study Assessing the Real-World Utilization of Elbasvir/ Grazoprevir (Zepatier) in Adult Patients with Chronic Hepatitis C (Z-PROFILE)	2016-2017	PI

Arbutus Biopharma Corporation	Evaluation ex vivo of Immunotherapeutics for chronic HBV	2017	PI
AbbVie	M16-127: A Multicenter, Open-Label, Study to Evaluate the Efficacy and Safety of Glecaprevir/ Pibrentasvir in Renally-Impaired Adults with Chronic Hepatitis C Virus Genotype 1 – 6 Infection	2017-2018	PI
Merck Sharp & Dohme Corp.	MK 3682-041: A Phase 2, Open-Label Clinical Trial to Study the Efficacy and Safety of 12 weeks of the Combination Regimen of MK-3682 + Ruzasvir in Subjects with Chronic Hepatitis C Virus (HCV) Genotype 1, 2, 3, 4, 5 or 6 Infection	2017-2018	PI
Merck Sharp & Dohme Corp.	MK 5172-106: Characterizing Risk Behaviour and Reinfection Rates for Successful Programs to Engage Core Transmitters in HCV Elimination (C-RESPECT)	2017-2019	PI
Janssen Research & Development, L. L. C.	A Phase 2b, Multicenter, Randomized, Open-label Study to Investigate the Efficacy, Safety and Pharmacokinetics of an 8- or 6-Week Treatment Regimen With Simeprevir, Odalasvir and AL-335 in Treatment-naïve and Treatment-experienced Subjects With Chronic Hepatitis C Virus Genotype 1, 2, 3, 4, 5 and 6 Infection, With and Without Cirrhosis (OMEGA-1)	2016-2018	PI
Janssen Research & Development, L. L. C.	A Prospective 3-Year Follow-up Study in Subjects Treated in a Preceding Phase 2 or 3 Study With a Regimen Containing Odalasvir and AL-335 With or Without Simeprevir for the Treatment of Hepatitis C Virus (HCV) Infection. (OMEGA-3)	2017-	PI
Janssen Research & Development, L. L. C.	A Phase 2a, Randomized, Partially-blind, Placebo-controlled Study to Assess the Efficacy, Safety, and Pharmacokinetics of 24 Weeks of Treatment With Multiple Doses of JNJ-56136379 as Monotherapy and in Combination With a Nucleos(t)ide Analog in Subjects With Chronic Hepatitis B Virus Infection (JADE)	2018-	PI
Sileagen L.L.C.	Collection of Plasma and Serum Samples from Individuals Initiating Therapy with FDA or Health Canada approved treatment such as Epclusa® (sofosbuvir/velpatasvir) or Mavyret™ (glecaprevir/pibrentasvir) for Chronic Hepatitis C Virus Infection for the Clinical Evaluation of a HCV Quantitative Assay	2017-2019	PI
Sileagen L.L.C.	Minimal Risk Specimen Collection Protocol	2017-	PI
ViiV Healthcare	204862: A Phase III, randomized, multicenter, parallel-group, non-inferiority study evaluating the efficacy, safety, and tolerability of switching to dolutegravir plus lamivudine in HIV-1 infected adults who are virologically suppressed (TANGO)	2018	PI
Janssen Research & Development, L. L. C.	A Phase 2b, Multicenter, Double-blind, Active-controlled, Randomized Study to Investigate the Efficacy and Safety of Different Combination Regimens Including JNJ-73763989 and/or JNJ-	2020-	PI

	56136379 for the Treatment of Chronic Hepatitis BVirus Infection (The REEF-1 Study)		
Gilead Sciences	A Phase 2/3 Study to Evaluate the Safety and Efficacyof Long Acting Capsid Inhibitor GS-6207 in Combination with an Optimized Background Regimen in Heavily Treatment Experienced People Living with HIV-1 Infection with Multidrug Resistance	2020-	PI
Merck Sharp & Dohme Corp.	A Phase 3 Randomized, Active-Controlled, Open-Label Clinical Study to Evaluate a Switch to Doravirine/Islatravir (DOR/ISL) Once-Daily in Participants With HIV-1 Virologically Suppressed on Antiretroviral Therapy	2020-	PI
Merck Sharp & Dohme Corp.	A Phase 3, Randomized, Active-Controlled, Double-Blind Clinical Study to Evaluate a Switch to Doravirine/Islatravir (DOR/ISL) Once-Daily in Participants with HIV-1 Virologically Suppressed onBictegravir/Emtricitabine/Tenofovir Alafenamide (BIC/FTC/TAF)	2020-	PI
Merck Sharp & Dohme Corp.	A Phase 3, Randomized, Clinical Study in HIV-1 Infected Heavily Treatment-Experienced ParticipantsEvaluating the Antiretroviral Activity of Blinded Islatravir (ISL), Doravirine (DOR), and Doravirine/Islatravir (DOR/ISL), Each Compared to Placebo, and the Antiretroviral Activity, Safety, and Tolerability of Open-Label DOR/ISL	2020-	PI
Research & Development Institute (RDI)	Prospective Blood Collection From Subjects With Confirmed Hepatitis B Infection	2020-	PI
ViiV Healthcare	212483: A Phase IIb, randomized, double blinded, parallel-group study to assess the efficacy, safety, tolerability, and resistance profile of GSK3640254 in combination with dolutegravir compared to dolutegravir plus lamivudine in HIV-1 infected, treatment-naïve adults (DYNAMIC)	2021-2022	PI
ACTG AIDS Clinical Trials Group	ACTIV-2/A5401: Adaptive Platform Treatment Trial for Outpatients with COVID-19 (Adapt Out COVID)	2021-2023	PI
Merck Sharp & Dohme Corp.	DRIVE: Canadian Real World Effectiveness of Doravirine and Doravirine/3TC/TDF, A Prospective Observational Cohort Study of Men and Women with HIV Infection with Anti-Retroviral Therapy	2021-	PI
Janssen Research & Development, L. L. C.	REEF-IT: A Phase 2, randomized, open-label, multicenter study to evaluate efficacy, pharmacokinetics, safety, and tolerability of response-guided treatment with JNJ-73763989 + JNJ-556136379 + nucleos(t)ide analog regimen with or without pegylated interferon alpha-2a in treatment-naïve patients with HBeAg positive chronic hepatitis B virus infection and normal ALT	2021-	PI

Janssen Research & Development, L. L. C.	E.mbrace: Randomized, Double-blind, Placebo-controlled, Multicenter Phase 3 Study to Assess the Efficacy, Safety And Immunogenicity of Vaccination With ExPEC9V in the Prevention of Invasive Extraintestinal Pathogenic Escherichia coli Disease in Adults Aged 60 Years And Older with a History of Urinary Tract Infection in the Past 2 Years	2021-	PI
AbbVie	M20-350: Acute Hepatitis C (HCV) Infection : Safety and Efficacy of Glecaprevir (GLE)/Pibrentasvir (PIB) 8 Week Treatment	2021-	PI
Gilead Sciences	GS-US-611-6273: A Phase 3, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of GS-5245 for the treatment of COVID-19 in participants with high -risk for disease progression (BIRCH)	2022-	PI
Pfizer Pharmaceuticals	C4671034: An Interventional Efficacy and Safety, phase 2, randomized, double blind, 3-arm study to investigate Nirmatrelvir/Ritonavir in nonhospitalized participants at least 12 years of age with symptomatic COVID-19 who are immunocompromised	2022-	PI
VIR	MARCH: A Phase 2 Study to Evaluate the safety, tolerability, and efficacy of regimens containing VIR-2218, VIR03434 and/or PEG-IFNa in Subjects with Chronic Hepatitis B Virus Infection	2023-	PI
ATEA	AT-01B-004: A PHASE 2, OPEN-LABEL STUDY TO ASSESS THE SAFETY AND EFFICACY OF BEMNIFOSBUVIR (BEM) AND RUZASVIR (RZR) IN SUBJECTS WITH CHRONIC HEPATITIS C VIRUS (HCV) INFECTION	2023-	PI

(1) Protocol Virologist

(B) Invited presentations (Last 5 years)

Oct 2015 14th Annual Interdisciplinary Forum in GI Medicine – Liver Forum – HCV: The Future

Oct 2015 4th International Symposium on Hepatitis Care in Substance Users – Treatment Paradigm for PWID

May 2016 Canadian Association for HIV Research Conference (CAHR), Winnipeg – The ART of Managing HIV/HCV Co-infection in the Era of New DAAs

Sep 2016 International Symposium on Hepatitis Care in Substance Users (INHSU), Oslo

Oct 2016 Symposium Speaker, Central and Eastern European Meeting on Viral Hepatitis and Co-infection with HIV Meeting, Bucharest - Overcoming the Challenges in Treating Different Patient Types

Apr 2017 European Association for the Study of the Liver (EASL), Amsterdam - Real-world utilization and effectiveness of elbasvir/grazoprevir in adult patients with chronic hepatitis C in Canada

Feb 2018 Canadian Symposium on HCV, CanHepC, Toronto

Mar 2018 Pain and Suffering Symposium, Vancouver

Mar 2018 Western Europe and Canada Advisory Board Meeting, Frankfurt, Germany – Using the Healthcare System to Vulnerable Populations

Apr 2018 EASL The International Liver Congress, Paris

- Four Symposia:

From Treatment Simplification to HCV Elimination
Shaping tomorrow together: from aspiration to achievement in HCV Care
HCV Unsymposium: Your Meeting Your Agenda
A Future Without Hep C: What will it take?

Apr 2018	European Virology meeting, Lisbon – Today's Populations at Risk: PWUD/OAT
Apr 2018	Speaker, MedReleaf Progress + Promise medical Cannabis Educational Initiatives, Medical Cannabis & HIV Symptom Management. Presentation: The use of cannabinoids in HIV-infected patients: towards an evidence-informed approach.
May 2018	Western Canada Advisory Board Meeting (Indivior OUD Regional Syntegration), Kelowna Western Canada Addiction Forum, Kelowna – HCV Treatment as a Tool to Address the Opioid Crisis
Jun 2018	Global Hepatitis Summit, Toronto - Three Symposia: HCV Elimination in Action: First Steps towards a Global Change Should we Treat HCV Patients with more Chaotic Lifestyles? Streamlining the Path to HCV Elimination Through Treatment Simplification - HCV and PWID: Not just about HCV
Sep 2018	Speaker, SLTC Canadian Summit
Feb 2019	Asian Pacific Association for the Study of the Liver, Manila – Sofosbuvir/velpatasvir (S/V) vs elbasvir/grazoprevir (E/G): is the non-pan-genotypic HCV treatment regimen dead?
Jun 2019	Gilead LEGA-C Meeting
Sep 2019	Workshop Facilitator, SLTC Canadian Summit. Presentation: Strategies to Enhance Screening and Linkage to Care for People Who Use Drugs
Feb 2020	Speaker, AbbVie HCV Elimination – Canadian Speaker Tour. Presentation: HCV Elimination: Strategies for Improving Outcomes in the PWID Population.
Oct 2022	French-Language Webinar on Monoclonal Antibodies for COVID-19 prevention
Oct 2022	The 10th International Network on Health and Hepatitis in Substance Users Conference
Dec 2022	Check:M.A.T.E Vaccine Webinar - Influenza
Feb 2023	Abbvie National Meeting 2023
Mar 2023	2023 AASLD North American Viral Hepatitis Elimination Summit
Mar 2023	Post-CROI HIV PeerToks
Apr 2023	London Drugs Conference
Apr 2023	Save-On-Foods Conference: Vaccination across Western Canada
Apr 2023	CSL Seqirus Canada Virtual Regional Consultancy Meeting

(C) Conference participation (Organizer, Keynote Speaker, etc.) (Last 5 years)

Nov 2015	Participant, American Association of Study of Liver (AASLD), The Liver Meeting, San Francisco
Jan 2016	Co-Chair, Ministerial Advisory Council on the Federal Initiative to Address HIV/AIDS (MAC-FI) Meeting, Ottawa
Feb 2016	Plenary, 5th Annual CanHepC Meeting and Symposium, Montreal
April 2016	Plenary Speaker, EASL The International Liver Congress, Barcelona
May 2016	Symposium Speaker, Canadian Association for HIV Research Conference (CAHR), Winnipeg – The ART of Managing HIV/HCV Co-infection in the Era of New DAAs
Sep 2016	Plenary, International Symposium on Hepatitis Care in Substance Users (INHSU), Oslo Oct
2016	Symposium Speaker, Central and Eastern European Meeting on Viral Hepatitis and Co-infection with HIV Meeting, Bucharest - Overcoming the Challenges in Treating Different Patient Types
Oct 2016	Symposium and Seminar Speaker, The Canadian Society of Addiction Medicine - LaSociete Medicale Canadienne sur l'Addiction (CSAM-SMCA) & International Collaboration in Addiction Medicine (ISAM) Annual Meeting, Montreal - C-EDGE CO STAR: risk of reinfection following successful therapy with Elbasvir (EBR)

and Grazoprevir (GZR) in persons who inject drugs (OPWID)receiving opioid agonist therapy (OAT)
- Advances in Pharmacotherapy – II

Nov 2016 Plenary, American Association for the Study of Liver Diseases Liver Meeting (AASLD), Boston

Apr 2017 Canadian Association for HIV Research Conference (CAHR), Montreal

Apr 2017	European Association for the Study of the Liver (EASL), Amsterdam - Real-world utilization and effectiveness of elbasvir/grazoprevir in adult patients with chronic hepatitis C in Canada
Sep 2017	Participant, International Symposium on Hepatitis Care in Substance Users (INHSU),Jersey City
Oct 2017	Participant, American Association of Study of Liver (AASLD), The Liver Meeting, Washington
Oct 2017	Participant, International Society of Addiction Medicine (ISAM), Abu Dhabi
Feb 2018	Canadian Symposium on HCV, Canadian Network on Hepatitis C, Toronto
Mar 2018	Participant, 31st Annual Pain and Suffering Symposium, Vancouver
Jun 2018	Participant, 2nd European HCV Policy Summit "Securing Sustainable Funding for HCV Elimination Plans", Brussels
Jun 2018	Plenary, CanHepC National Stakeholder Workshop, Toronto – A Blueprint for a HCV National Action Plan
Jun 2018	Symposia Speaker, Global Hepatitis Summit, Toronto
Sep 2019	Symposium Speaker, AbbVie Symposium at INHSU, Montreal
Nov 2019	Symposium Organizer and Speaker, ISAM, New Delhi

SERVICE COMMITMENTS

Member, Health Canada National Advisory Committee on the Future of AIDS Research (1995-97)
Consultant, Western Canadian NRC Consultative Committee on Industry/Academic Research Initiatives (1998)
Consultant, Canadian International Development Agency – Development of HIV Treatment Programs in Guyana (2002-2004)

SERVICE TO THE COMMUNITY

(A) Memberships on scholarly societies, including offices held and dates
Chairman, Canadian Infectious Diseases Society Program Planning Committee (1991-96)
Member, Royal College Scientific Program Committee (1991-97)
Councillor, Canadian Association for HIV Research (1993-96)
Member, Royal College Academic-Industry Liaison Committee (1995-97)
Member, Health Canada National HIV Immunology Advisory Committee (1996-2000)
Secretary, Canadian Association for HIV Research (2001-2004)
Councillor, Canadian Infectious Diseases Society (2002-2004)
Chair Elect, Canadian Pediatric AIDS Research Group (2003-2004)
Chair, Canadian Pediatric AIDS Research Group (2004-2005)
President Elect, Canadian Association for HIV Research (2004-2007)
President, Canadian Association for HIV Research (2007-2009)
Co-Chair, Ministerial Advisory Council on the Federal Initiative (MAC-FI) to Address HIV/AIDS, HCV, and Related Health Conditions in Canada (2007 – 2018)

(B) Memberships on other committees, including offices held and dates
Vice-President, Union Affairs, Fédération des Médecins Résidents & Internes du Québec (1983-86)
Vice-President, Professional Association of Residents and Interns of Manitoba (1986-87)
Chairman, AIDS Outreach Committee, St.-John's Anglican Church, Ottawa (1990-94)
Board member, Ottawa-Carleton Council on AIDS (1993-94)
Member, British Columbia Ministry of Health Advisory Committee on HIV/AIDS (1998-2001)
Board Member, Societé Santé en Français (2003-2005)
President, Société Santé en Français (2007-2012)
President, RésoSanté Colombie-Britannique (2004-2007; 2012 – Present)

EDITORSHIPS

Editorial Board, Journal of Acquired Immune Deficiency Syndromes (1999 – Present)
Co-Chair, HIVResistanceWeb.com academic website (2000-2003)
Editorial Board, AIDS (2001-present)
Assistant Editor, Clinical Sciences, Journal of Acquired Immune Deficiency Syndromes (2003-2009)
HIV Editor, MedScape (2003 – Present)
Editor, Side Effects Management, TheBody.Com (2003-2009)
Editorial Advisory Panel, Future Virology (2010-Present)
Editorial Board, ARC Journal of Hepatology and Gastroenterology (2015 – Present)
Editorial Advisory Board, Clinical Infectious Diseases (2017-Present)

AWARDS AND DISTINCTIONS

McConnell Scholarship, McGill University (1978, tuition award)
University Scholar, McGill University (1978-82, top 15/160 standing, medical school class)
Research Fellowship, Medical Research Council of Canada (1988-90, $40000/year)
Ontario Ministry of Health Career Scientist Award (1991-93, $50000/year)
Canadian Infectious Disease Society Young Investigator Award (1992, $15000)
Canadian Association for HIV Research Young Investigator Award (1992, $2000)
International Society for Antiviral Research Young Investigator Award (1993, $1200)
St-Paul's Hospital Department of Medicine Hoffman Research Award (1995)

(A) Award for service (Indicate name of award, awarding organizations, and date)

Honorable mention, Logie Medical Ethics Award (1982, A Christian physician's view of euthanasia)
Award of Excellence in HIV Care & Research, Canadian Hemophilia Society (1998)
Prix Napoléon Gareau, La Fédération des francophones de la Colombie-Britannique (2007)
Queen Elizabeth II Diamond Jubilee Medal of Recognition for contribution to the people of Canada in HIV/AIDS (2012)
AccolAIDS for Science/Research/Technology, Positive Living BC (2014)
Journée de la francophonie 2016/ Contribution to the provision of Health services in French in British Columbia/ The B.C. Government (2016)
Hepatitis Elimination Champions 2020/ Coalition for Global Hepatitis Elimination (2020)

PUBLICATIONS

(A) Referred publications

1. Hammond GW, Buchanan D, Malazdrewicz R, Conway B, Tate R, Sekla L, Fast M, Ronald AR and the Manitoba AIDS Virus Epidemiology Study (MAVES) group. Seroprevalence and demographic information of patients at risk for human immunodeficiency virus (HIV) infection in Manitoba. *J Acq Imm Def Syn* 1(2):138-142, April 1988.
2. Conway B, Zhanel GG, Ronald AR. The value of single dose therapy to diagnose the site of urinary infections. *Intern J Exp Clin Chemother* 2:39-47, 1989.
3. Ronald AR, Conway B, Zhanel GG. The value of single dose therapy to diagnose the site of urinary infections. *Chemotherapy* 36(Suppl 1):2-9, 1990.
4. Conway B. Choosing interns: an exercise in frustration. *Can Med Assoc J* 140:359, 1989.

5. Conway B, Halliday WC, Brunham RC. Human immunodeficiency virus (HIV)-associated progressive multifocal leuko-encephalopathy: apparent response to 3'-azido-3'-deoxythymidine (AZT). *Rev Infect Dis* 12:479-483, 1990.
6. Conway B, Tomford WW, Hirsch MS, Schooley RT, Mankin HJ. Effects of gamma irradiation on HIV-1 in a bone allograft model. *Trans Orthop Res Soc* 15:225, 1990.
7. Conway B, Adler KE, Bechtel LJ, Kaplan JC, Hirsch MS. Detection of HIV-1 DNA in crude cell lysates of peripheral blood mononuclear cells by the polymerase chain reaction and non-radioactive oligonucleotide probes. *J Acq Imm Def Synd* 3(11):1059-1064, November 1990.
8. Conway B, Tomford WW, Mankin HJ, Hirsch MS, Schooley RT. Radiosensitivity of HIV-1 - potential application to sterilization of bone allografts. *AIDS* 5(5):608-609, 1991.
9. Sahai J, Conway B, Cameron D, Garber G. Zidovudine-associated hypertrichosis and nail pigmentation in an HIV-infected patient. *AIDS* 5:1395-1396, 1991.
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204. Conway B, Rashidi B, Hakobyan B, Sharma S, Alenezi O, Hung A, Yen K, Huang X, Tossonian H. Identifying HCV treatment barriers amongst high risk population of Vancouver Downtown Eastside. November 7-1. AASLD Annual Meeting. Boston, USA. 2014
205. Tossonian H, Hakobyan S, Wang J, Sharma S, Alenezi O, Conway B. Long-term follow-up of HIV-infected people who inject drugs on once-daily combination antiretroviral therapy. Poster Presentation. 23rd Annual Canadian Conference on HIV/AIDS Research, May 1-4. St. John's, Newfoundland, Canada.2014.
206. Tossonian H, Hakobyan S, Sharma S, Kim A, Conway B. Simplified Antiretroviral Therapy with Stribild & Darunavir for Treatment-Experienced Patients. The 24th Annual Canadian Conference on HIV/AIDS Research. April 30 -May 3. Toronto, Ontario, Canada. 2015.
207. Conway B, Sharma S, Hakobyan S, Rashidi B, Tahmasebi S, Tossonian H. Identifying HCV treatment barriers as part of community portable pop-up clinics. The 24th Annual Canadian Conference on HIV/AIDS Research. April 30 -May 3. Toronto, Ontario, Canada. 2015.
208. Conway B, Hakobyan S, Kerkerian G, Tahmasebi S, H. Tossonian. Community-Based Infectious Disease Clinics: A Tool of Engagement for Vulnerable Populations. CPS ID meeting. The 24th Annual Canadian Conference on HIV/AIDS Research. April 30 -May 3. Toronto, Ontario, Canada. 2015
209. Conway B, Hakobyan S, Sharma S, Tossonian H. HIV-Infected PWID: Engagemgent In The Cascade Of Care. Conference on Retroviruses and Opportunistic Infections (CROI). February 23-26. Seattle, Washington, USA. 2015.
210. Conway B, Hakobyan S, Kerkerian G, Sharma S, Tahmasebi S, Zahedieh F, Pagarigan H, Tossonian H. Community-Based Infectious Disease Clinics: A Tool of Engagement for Vulnerable Populations. Annual Medical Microbiology and Infectious Diseases Conference (AMMI Canada – CACMID). April 16-18. Charlottetown, PEI, Canada. 2015
211. Hakobyan S, Tossonian H, Sharma S, Mohiuddin S, Zahedieh F, Kerkerian G, Krausz M, Conway B. Treat Or Do Not Treat Vulnerable Inner City Population With Concurrent Disorders For Chronic Hepatitis C? Oral Presentation. Canadian Psychiatric Association Annual Conference. October 1-3. Vancouver, British Columbia, Canada. 2015.
212. Conway B, Hakobyan S, Sharma S, Rashidi B, Tossonian H. Identifying HCV Treatment Barriers Amongst High Risk Population of Vancouver Downtown Eastside. 4th Canadian Symposium on HCV. February 27. Banff, British Columbia, Canada. 2015

213. Hakobyan S, Sharma S, Tossonian H, Conway B. Cascade of Care of HIV/HCV Co-Infected Patients in Downtown East Side of Vancouver. 11th International Workshop on HIV & Hepatitis Co-infection. 11 – 12 June. London, United Kingdom. 2015.
214. Conway B, Hakobyan S, Sharma S, Zahedieh F, Mohiuddin F, Tossonian H. Correlates of Successful HCV Treatment in HIV Co-Infected Vulnerable Populations. 11th International Workshop on HIV & Hepatitis Co-infection. 11 – 12 June. London, United Kingdom. 2015.
215. Sharma S, Hakobyan S, Mohiuddin S, Tossonian H, Conway B. Changes to Antiretroviral Therapy while on HCV Treatment in HIV/HCV Co-Infected Patients. 11th International Workshop on HIV & Hepatitis Co-infection. 11 – 12 June. London, United Kingdom. 2015.
216. Hakobyan S, Sharma S, Zahedieh F, Tossonian H, Conway B. A Multidisciplinary Approach to Engagement in Care of HIV-Infected Individuals with Unstable Housing. Oral Presentation. The North American Housing and HIV/AIDS Research Summit VIII: Tackling the Social Drivers of HIV. September 14-16. Washington DC, USA. 2015.
217. Hakobyan S, Sharma S, Zahedieh F, Tossonian H, Conway B. HIV Infection Risk in Homeless Participants in Community Pop-Up Clinics. Oral Presentation. The North American Housing and HIV/AIDS Research Summit VIII: Tackling the Social Drivers of HIV. September 14-16. Washington DC, USA. 2015.
218. Hakobyan S, Vafadary S, Zahedieh F, Sharma S, King A, Tossonian H, Conway B. HCV Re-infection in High-Risk People Who Inject Drugs. 4th International Symposium on Hepatitis C Care in Substance Users. Poster Presentation. October 7-9. 2015. Sydney. Australia.
219. Alimohammadi A, Sharma S, Hakobyan S, Hsieh YL, Tossonian H, King A, Conway B. Evaluation of Treatment of HCV Infection in Active Injection Drug Users. Oral Presentation. 4th International Symposium on Hepatitis C Care in Substance Users. October 7-9. Sydney. Australia. 2015.
220. Hakobyan S, Sharma S, Zahedieh F, Vafadary S, King A, Tossonian H, Conway B. The Community Pop-Up Clinic as a Tool of Engagement for Vulnerable Populations With HCV And HIV Infection. Sydney. Australia, October 7-9. Poster Presentation. 4th International Symposium on Hepatitis C Care in Substance Users. October 7-9. Sydney. Australia. 2015.
221. Conway B, Hakobyan S, Sharma S, King A, Tossonian H. HCV HIV Co-Infected PWID Treated for HCV at Inner City Clinic. Oral Presentation. 4th International Symposium on Hepatitis C Care in Substance Users. , October 7-9. Sydney. Australia. 2015.
222. Hakobyan S, Vafadary S, Raycraft T, Sharma S, Conway B. Correlates Of Successful HCV Treatment In HIV Co-Infected Vulnerable Populations. Poster Presentation. 15th European Aids Conference. October 21-24, Barcelona Spain. 2015
223. Hakobyan S, Vafadary S, Sharma S, Kiani G, Conway B. Characteristics Of HIV Positive Patients Lost To Follow-Up In An Infectious Disease Clinic In Downtown Vancouver. Oral Presentation. Gay Men Health Summit, Summit 2015: Undoing Stigma, November 5 & 6, Vancouver, BC, Canada. 2015.
224. Vafadary S, Hakobyan S, Kiani G, Raycraft T, Sharma S, Conway B. The Cascade Of Care Of HIV-Positive Men Who Have Sex With Men In The Vancouver Downtown East Side. Oral Presentation. Gay Men Health Summit, Summit 2015: Undoing Stigma, November 5 & 6, Vancouver, BC, Canada. 2015.
225. Hakobyan S, Vafadary S, Sharma S, Kiani G, Raycraft T, Conway B. Characteristics Of HIV Positive Patients Who Were Lost To Follow-Up In The Inner City. Oral Presentation. Where Is The Patient Voice, Nov 12-14, Vancouver, BC, Canada., 2015
226. Conway B, Hakobyan S, King A, Zahedieh F, Tossonian H, Vafadary S, Sharma S. Correlates of Successful HCV Treatment in HIV Co-Infected Vulnerable Populations. American Association of Study of Liver (AASLD). The Liver Meeting. November 13-17. San Francisco, USA, 2015
227. Hakobyan S, Sharma S, King A, Zahedieh F, Alimohammadi A, Tossonian H, Conway B. Cascade of Care of HCV & HIV Infected Patients Identified Through Community Pop-Up Clinics (CPCs). Poster Presentation. American Association of Study of Liver (AASLD). The Liver Meeting. November 13-17. San Francisco, USA, 2015.
228. Liu Y, Luo Y, Liu X, Sullivan D, Juday TR, Conway B. Better Work Productivity and Activity in Patients on Ombitasvir/ Paritaprevir/Ritonavir and Dasabuvir with or without Ribavirin (3D+RBV or 3D) in

Treatment-Naïve Adults with Genotype 1(GT1) Chronic Hepatitis C. American Association of Study of Liver (AASLD). The Liver Meeting. November 13-17. San Francisco, USA, 2015.

229. Conway B, Luo Y, Arama V, Ghesquiere W, Sasadeusz J, Skoien R, Soza A, Inglot M, Sullivan D, Podsadecki T, Dore G. Ombitasvir/Paritaprevir/r and Dasabuvir +/- Ribavirin is Better Tolerated Than Telaprevir + Peginterferon/Ribavirin With Less Frequent and Less Severe Anemia in the MALACHITE-I and MALACHITE-II Trials. American Association of Study of Liver (AASLD). The Liver Meeting. November 13-17. San Francisco. USA, 2015.
230. Alimohammadi A, Sharma S, Hakobyan S, Conway B. Evaluation of Treatment of HCV Infection in Active Injection Drug Users. CDDW Canadian Digestive Diseases Week (CDDWTM) Meeting. Poster Presentation. 26-29 Feb, Montreal, QC, Canada, 2016.
231. Conway B, Vafadary S, Raycraft T, Zahedieh F, Sharma S, Kiani G, Alimohammadi A, Hakobyan S. HCV Re-Infection in High-Risk People who Inject Drugs. CDDW Canadian Digestive Diseases Week (CDDWTM) Meeting. Poster Presentation. 26-29 Feb, Montreal, QC, Canada, 2016.
232. Conway B, Vafadary S, Sharma S, Zahedieh F, Shravah J, Hakobyan S. The Community Pop-Up Clinic as a Tool of Engagement for Vulnerable Populations with HCV and HIV Infections. CDDW Canadian Digestive Diseases Week (CDDWTM) Meeting. Poster Presentation. 26-29 Feb, Montreal, QC, Canada, 2016.
233. Hakobyan S, Vafadary S, Sharma S, Raycraft T, Conway B. HCV Treatment of HIV-HCV Co-Infected PWID at a Tertiary Clinic. CDDW Canadian Digestive Diseases Week (CDDWTM) Meeting. Poster Presentation. 26-29 Feb, Montreal, QC, Canada, 2016.
234. Raycraft T, Hakobyan S, Vafadary S, Sharma S, Conway B. HCV Re-Infection in High-Risk People Who Inject Drugs. Poster Presentation. European Association of Study of Liver. EASL. April 13-17. Barcelona, Spain, 2016.
235. Hakobyan S, Tossonian H, Sharma S, Raycraft T, Vafadary S, Zahedieh F, Kerkerian G, Krausz M, Conway B. HCV Treatment in Vulnerable Inner City Population With Concurrent Disorders. Poster Presentation. European Association of Study of Liver. EASL. April 13-17. Barcelona, Spain, 2016.
236. Hakobyan S, Vafadary S, Raycraft T, Kiani G, Sharma S, Conway B. Stribild and Darunavir Evaluation (Stride): A New Standard In Salvage Therapy. Oral Presentation. Canadian Association for Clinical Microbiology and Infectious Diseases. AMMI-CACMID. Mar 30-Apr 2. Vancouver, BC, Canada.Vancouver, BC, Canada, 2016.
237. Vafadary S, Hakobyan S, Raycraft T, Kiani G, Sharma S, Conway B. A Retrospective Observational Cohort Study of HCV Re-Infection In High-Risk People Who Inject Drugs. Poster Presentation. Canadian Association for Clinical Microbiology and Infectious Diseases. AMMI-CACMID. Mar 30-Apr 2. Vancouver, BC, Canada.Vancouver, BC, Canada. 2016.
238. Raycraft T, Hakobyan S, Sharma S, Vafadary S, Kiani G, Alimohammadi A, Conway B. Factors Leading to Regimen Change in HIV-Positive Patients. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
239. Raycraft T, Hakobyan S, Sharma S, Vafadary S, Kiani G, Alimohammadi A, Conway B. Characteristics of HIV-Positive Patients Requiring Salvage Therapy in Downtown Vancouver, BC. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
240. Vafadary S, Hakobyan S, Kiani G, Raycraft T, Conway B. Characteristics of HIV Positive Patients Who Were Lost To Follow-Up in the Inner City. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
241. Vafadary S, Hakobyan, S, Raycraft T, Kiani G, Conway B. Stribild: A Promising New Treatment for Suppression of HIV Viral Load in People Who Inject Drugs. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
242. Conway B, Kiani G, Vafadary S, Raycraft T, Shravah J, Hakobyan S. Integrase Inhibitor-Based Antiretroviral Therapy In Vulnerable Populations. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
243. Conway B, Vafadary S, Raycraft T, Shravah J, Kiani G, Alimohammadi A, Hakobyan S. HCV Re-Infection In High-Risk HIV Co-Infected People Who Inject Drugs In A Multidisciplinary Clinic. Poster

Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.

244. Hakobyan S, Vafadary S, Raycraft T, Kiani G, Conway B. The Community Pop-Up Clinic as a Tool of Engagement for Vulnerable Populations with HIV and HCV Infection. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
245. Hakobyan S, Vafadary S, Kiani G, Raycraft T, Conway B. HCV Treatment Of HIV-HCV Co-Infected PWID at a Tertiary Clinic. Poster Presentation. The Canadian Association for HIV Research Annual Conference, CAHR, May 12-15 Winnipeg, MB, Canada, 2016.
246. Conway B, Hakobyan S, Raycraft T, Bhutani Y, Alimohammadi A, Shahi R, Kiani G, Vafaday S. Analysis of Adherence to All-Oral HCV Therapy in a Cohort of People Who Inject Drugs (PWID). Poster Presentation. 5th International Symposium on Hepatitis Care in Substance Users (INHSU), September 7-9, Oslo, Norway, 2016.
247. Hakobyan S, Raycraft T, Vafadary S, Alimohammadi A, Bhutani Y, Kiani G, Conway B. Efficacy of All-Oral HCV Therapy in People Who Inject Drugs. Poster Presentation. 5th International Symposium on Hepatitis Care in Substance Users (INHSU), September 7-9, Oslo, Norway, 2016.
248. Kiani G, Vafadary S, Raycraft T, Hakobyan S, Conway B. Portable Pop-Up Clinics: Characteristics of Engagement in Care, An Observational Prospective Cohort Study. Poster Presentation. 5th International Symposium on Hepatitis Care in Substance Users (INHSU), September 7-9, Oslo, Norway, 2016.
249. Alimohammadi A, Hakobyan S, Raycraft T, Shahi R, Conway B. Recurrent HCV Viremia in People Who Actively Inject Drugs. Poster Presentation. 5th International Symposium on Hepatitis Care in Substance Users (INHSU), September 7-9, Oslo, Norway, 2016.
250. Raycraft T, Hakobyan S, Vafadary S, Alimohammadi A, Shahi R, Conway B. Comparison of Rates of Recurrent Viremia in High-Risk Patients Receiving All-Oral and Interferon-Based Regimens. Oral Presentation. 5th International Symposium on Hepatitis Care in Substance Users (INHSU), September 7-9, Oslo, Norway, 2016.
251. Hakobyan S, Vafadary S, Raycraft T, Sharma S, Conway B. HCV Treatment of HIV-HCV Co-Infected PWID at a Tertiary Clinic. Canadian Digestive Diseases Week, February 26-29, Montreal, Canada, 2016.
252. Kiani G, Shahi R, Alimohammadi A, Raycraft T, Singh A, Conway B. Community pop-up clinic: a harm reduction strategy to engage hepatitis C virus infected injection drug users. Canadian Digestive Diseases Week, February 26-29, Montreal, Canada, 2016.
253. Conway B, Vafadary S, Raycraft T, Zahedieh F, Sharma S, Hakobyan S. HCV Re-Infection in High-Risk People Who Inject Drugs. Canadian Digestive Diseases Week, February 26-29, Montreal, Canada, 2016.
254. Conway B, Cooper C, Bruneau J, Feld J, Deshaies L, Fraser C, Macphail G, Powis J, Steingart C, Stewart K, Tomas R, Webster D,Cox J, Drolet M, Mcgovern M. Characterization of HCV Infected PWID in the Setting of Clinical Care in Canada (CAPICA): A Retrospective Study. Canadian Digestive Diseases Week, February 26-29, Montreal, Canada, 2016.
255. Conway B, Janczewska E, Luo Y, Curescu M, Greenbloom S, Streinu-Cercel A, Caruntu FA, Ghesquiere W, Knysz B, Mazur W, Fuster F, Motoc A, Soza A, Arama V, Dalgard O, Sullivan D, Liu X, Podsadecki T. Malachite-I: Phase 3B Trial of ombitasvir/paritaprevir/r and dasabuvir +/− ribavirin or telaprevir + peginterferon/ribavirin in treatment-naïve adults with HCV genotype 1. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.
256. Alimohammadi A, Raycraft T, Shahi R, Singh A, Kiani G, Conway B. Using All-Oral Hepatitis C Virus Therapy to Reduce Recurrent Viremia and Increase SVR. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.
257. Singh A, Alimohammadi A, Raycraft T, Shahi R, Kiani G, Conway B. The evaluation of homelessness on HCV treatment outcomes among current and former people who inject drugs. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.

258. Kiani G, Shahi R, Alimohammadi A, Raycraft T, Singh A, Conway B. Community pop-up clinic: a harm reduction strategy to engage hepatitis C virus infected injection drug users. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.
259. Alimohammadi A, Singh A, Shahi R, Raycraft T, Kiani G, Conway B. HCV-infected people who inject drugs (PWID): engagement in care and treatment, and prevention of reinfection. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.
260. Truong D, Bhutani Y, Singh A, Nitulescu R, Wong L, Alimohammadi A, Klein M, Holeksa J, Conway B. Reversal of Fibrosis Following Successful HCV Therapy among HIV/HCV Co-Infected People who Use Drugs. Poster Presentation. 6th International Symposium on Hepatitis Care in Substance Users, September 5-8, Jersey City/New York, USA, 2017.
261. Alimohammadi, Singh A, Shahi R, Raycraft T, Kiani G, Conway B. HCV-infected People Who Inject Drugs (pwid): Engagement in Care and Treatment, and Prevention of Reinfection. Poster Presentation. EASL International Liver Conference, 19-23 April, Amsterdam, The Netherlands, 2017.
262. Kiani G, Alimohammadi A, Amiri N, Raycraft T, Truong D, Conway B. Comorbidities among HIV/HCV Coinfected People who Inject Drugs (PWID): A Retrospective Cohort Study. Poster Presentation. 6th International Symposium on Hepatitis Care in Substance Users. September 6-8, Jersey City/New York City, United States, 2017.
263. Conway B, Truong D, Bhutani Y, Raycraft T, Kiani G, Alimohammadi A. HCV-Infected People who Inject Drugs (PWID): Long-term Engagement to Prevent Recurrent Viremia (RV) in Patients that Received IFN-Based Therapies. Poster Presentation. 6th International Symposium on Hepatitis Care in Substance Users. September 6-8, Jersey City/New York City, United States, 2017.
264. Holeksa J, Alimohammadi A, Bassi A, Wong L, Nitulescu R, Klein M, Conway B. Fibrosis reversal in HCV/HIV co-infected people who inject drugs (PWID) after successful HCV treatment. Poster Presentation. 7th Canadian Symposium on HCV/Canadian Liver Meeting, Feb 9-11, Toronto, Canada, 2018.
265. Alimohammadi A, Bassi A, Holeksa J, Bhutani Y, Yung R, Thiam A, Conway B. All-oral anti-HCV therapy in injection drug users: Updated real world data. Oral Presentation. 7th Canadian Symposium on HCV/Canadian Liver Meeting, Feb 9-11, Toronto, Canada, 2018.
266. Alimohammadi A, Holeksa J, Bassi A, Thiam A, Conway B. Treating HCV in people who inject drugs with elbasvir/grazoprevir. Poster Presentation. EASL Monothematic Conference: Striving towards the elimination of HCV infection. February 2-3, Berlin, Germany, 2018.
267. Holeksa J, Alimohammadi A, Bassi A, Tham A, Conway B. Is macro-elimination of HCV infection the right approach for Canada? Poster Presentation. EASL Monothematic Conference: Striving towards the elimination of HCV infection. February 2-3, Berlin, Germany, 2018.
268. Holeksa J, Alimohammadi A, Bassi A, Thiam A, Conway B. Thu-084 - Is Macro-Elimination of HCV Infection the Right Approach for Canada? Poster Presentation. EASL International Liver Conference, 11-15 April. Paris, France, 2018.
269. Holeksa J, Bassi A, Alimohammadi A, Thiam A, Conway B. HCV Treatment Responses Among People Who Use Drugs: an Evaluation of Patients on and Off Opiate Agonist Therapy in a Real-Life Setting." Poster Presentation. EASL International Liver Conference, 11-15 April. Paris, France, 2018.
270. Alimohammadi A, Holeksa J, Bassi A, Bhutani Y, Thiam A, Conway B. Recurrent Viremia After Successful Hepatitis C Virus Therapy with Direct-Acting Antivirals in a Cohort of People Who Use Drugs. Poster Presentation. EASL International Liver Conference, 11-15 April. Paris, France, 2018.
271. Alimohammadi A, Holeksa J, Bhutani Y, Thiam A, Bassi A, Conway B. A Comparison of Elbasvir/grazoprevir, Ledispasvir/sofosbuvir and Velpatisvir/sofosbuvir Therapy Among People Who Use Drugs (PWUD): Real World Experience. Poster Presentation. EASL International Liver Conference, 11-15 April. Paris, France, 2018.
272. Conway B, Tam E, Tremblay J, Fraser C, Ramji A, Borgia S, Tsoi K, Yoshida E, Rajendran B, Stewart K, Macphail G, Trottier B, Ghali P, Halsey-Brandt J, Trepanier J. Z-PROFILE: Real-world utilization and effectiveness of elbasvir/ grazoprevir in adult patients with chronic hepatitis C in Canada. Poster Presentation. EASL International Liver Conference, 11-15 April. Paris, France, 2018.

273. Alimohammadi A, Holeksa J, Thiam A, Conway B. Co-morbidities among HIV-Infected Individuals. Poster Presentation. 27th Annual Canadian Conference on HIV/AIDS Research. April 26-29, Vancouver, Canada, 2018.
274. Holeksa J, Alimohammadi A, Thiam A, Conway B. Community-Based Identification of HIV-Infected Individuals: A Focus on Vulnerable Populations. Oral Presentation. 27th Annual Canadian Conference on HIV/AIDS Research. April 26-29, Vancouver, Canada, 2018.
275. Holeksa J, Alimohammadi A, Conway B. Hepatitis C (HCV) Treatment in People Who Inject Drugs (PWID): A Comparison of Single-and Multi-Tablet. Oral Presentation. Association of Medical Microbiology and Infectious Disease Canada. May 2-5, Vancouver, Canada, 2018.
276. Holeksa J, Alimohammadi A, Truong D, Conway B. Towards HCV micro-elimination: why HCV is not like HIV. Poster Presentation. 16th ISVHLD Global Hepatitis Summit, June 14-17, Toronto, Canada, 2018.
277. Alimohammadi A, Holeksa J, Truong D, Conway B. Using rapid point-of-care HCV testing to engage marginalized inner-city populations. Poster Presentation. 16th ISVHLD Global Hepatitis Summit, June 14-17, Toronto, Canada, 2018.
278. Alimohammadi A, Holeksa J, Thiam A, Conway B. Co-mordibities among HIV-infected individuals. Poster Presentation. 14th International Workshop on Co-infection. May 16-18, Seville, Spain, 2018.
279. Holeksa J, Bassi A, Alimohammadi A, Nitulescu R, Wong L, Klein M, Turong D, Conway B. Fibrosis reversal in HCV/HIV co-infected people who inject drugs (PWID) after successful HCV treatment. 14th International Workshop on Co-infection. Poster presentation. May 16-18, Seville, Spain, 2018.
280. Thiam A, Alimohammadi A, Holeksa J, Yung R, Truong D, Conway B. A comparison of single and multiple tablet regimens for the treatment of HCV infection among HIV co-infected individuals. 14th International Workshop on Co-infection. Oral presentation. May 16-18, Seville, Spain, 2018.
281. Conway B, Alimohammadi A, Holeksa J, Truong D. Elbasvir/grazoprevir, ledipasvir/sofosbuvir and velpatisvir/sofosbuvir therapy among people who use drugs (PWUD): Real world experience. Poster Presentation. The 7th International Symposium on Hepatitis Care in Substance Users. September 19-21, Cascais, Portugal, 2018.
282. Holeksa J, Alimohammadi A, Thiam A, Truong D, Conway B. Re-infection and attending SVR12. Poster presentation. The 7th International Symposium on Hepatitis Care in Substance Users. September 19-21, Cascais, Portugal, 2018.
283. Holeksa J, Alimohammadi A, Truong D, Conway B. Community Outreach Events- Engaging the disengaged. Oral presentation. The 7th International Symposium on Hepatitis Care in Substance Users. September 19-21, Cascais, Portugal, 2018.
284. Truong D, Holeksa J, Alimohammadi A, Thiam A, Conway B. Reversal of Fibrosis after successful HCV treatment in people who inject drugs (PWID). Poster presentation. The 7th International Symposium on Hepatitis Care in Substance Users. September 19-21, Cascais, Portugal, 2018.
285. Thiam A, Alimohammadi A, Holeksa J, Yung R, Truong D, Conway B. Loss to follow-up among PWID receiving HCV treatment: predictors and intervention strategies. Poster presentation. The 7th International Symposium on Hepatitis Care in Substance Users. September 19-21, Cascais, Portugal, 2018.
286. Thiam A, Alimohammadi A, Holeksa J, Yung R, Truong D, Conway B. Impact of homelessness on active drug use and successful HCV therapy: a mixed methods analysis. Oral presentation. The Canadian Society of Addiction Medicine conference. October 25-27, Vancouver, Canada, 2018.
287. Alimohammadi A, Holeksa J, Thiam A, Truong D, Conway B. HCV therapy for people who use drugs, delivered in a multidisciplinary setting. Poster presentation. The 20th International Society of Addiction Medicine Annual Meeting, November 3-6, Busan, Republic of Korea, 2018.
288. Holeksa J, Alimohammadi A, Thiam, A., Truong, D, Conway B. Community-Based Identification of HCV-Infected People Who Use Drugs (PWUD). Poster presentation. The 20th International Society of Addiction Medicine Annual Meeting, November 3-6, Busan, Republic of Korea, 2018.
289. Thiam A, Alimohammadi A, Holeksa J, Yung R, Truong D, Conway B. Lost to follow-up among PWID in pre and post SVR HCV treatment and how to mitigate it. Oral presentation. The 20th International Society of Addiction Medicine Annual Meeting, November 3-6, Busan, Republic of Korea, 2018.

290. Holeksa J, Truong D, Alimohammadi A, Conway B. Assessing for improvement in synthetic and metabolic liver function post-SVR. Poster presentation. American Assocation for the Study of Liver Diseases. Nov 9-13, San Francisco, USA, 2018.
291. Holeksa J, Magel T, Thiam A, Chu L, Yung R, Truong D, Conway B. Preliminary Results from a Novel Model of HCV Care in Opiate Substitution Therapy (OST) Clinics: Find 50. Poster presentation. American Assocation for the Study of Liver Diseases/European Association for Study of the Liver HCV Special Conference. February 1-2, Miami, Florida, USA, 2019.
292. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway B. A Longitudinal Evaluation of Treatment of HCV Infection Among People Who Do Drugs (PWUD). Poster presentation. American Assocation for the Study of Liver Diseases/European Association for Study of the Liver HCV Special Conference. February 1-2, Miami, Florida, USA, 2019.
293. Thiam A, Alimohammadi A, Holeksa J, Magel T, Yung R, Truong D, Conway B. Impact of age on successful HCV therapy among active drug users. Poster presentation. American Assocation for the Study of Liver Diseases/European Association for Study of the Liver HCV Special Conference. February 1-2, Miami, Florida, USA, 2019.
294. Thiam A, Alimohammadi A, Holeksa J, Magel T, Yung R, Truong D, Conway B. Homelessness impact on active drug use among hepatitis C infected population. Poster presentation. American Assocation for the Study of Liver Diseases/European Association for Study of the Liver HCV Special Conference. February 1-2, Miami, Florida, USA, 2019.
295. Holeksa J, Magel T, Truong D, Thiam A, Yung R, Chu L, Conway B. Prevention of HIV transmission and optimization of HIV therapy among HCV-infected people who inject drugs (PWID) by engagement in long-term medical care. Poster presentation. Asian Pacific Association for the Study of the Liver. February 20-24, Manila, Philippines, 2019.
296. Conway B, Truong D, Magel T, Thiam A, Chu L, Yung R, Holeksa J. Sofosbuvir/velpatasvir (S/V) vs elbasvir/grazoprevir (E/G): is the non-pan-genotypic HCV treatment regimen dead? Poster presentation. Asian Pacific Association for the Study of the Liver. February 20-24, Manila, Philippines, 2019.
297. Holeksa J, Magel T, Thiam A, Truong D, Yung R, Chu L, Conway B. Long-term follow up of an inner-city cohort treated for hepatitis C virus (HCV) infection in the interferon (IFN) and all-oral (DAA) eras: adding to the rationale for emergent HCV treatment. Poster presentation. International Workshop on HIV and Hepatitis Observational Databases. March 28-30, Athens, Greece, 2019.
298. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway B. Engagement in multidisciplinary care: a new tool to address the opioid crisis. Poster presentation. Western Canada Addiction Forum. May 3-5, Kelowna, Canada, 2019.
299. Holeksa J, Magel T, Reyes-Smith L, Torshizi O, Thiam A, Chu L, Yung R, Truong D, Conway B. Preliminary results from a novel model of HCV care in OST clinics: find 50. Poster presentation. Western Canada Addiction Forum. May 3-5, Kelowna, Canada, 2019.
300. Holeksa J, Magel T, Thiam A, Chu L, Yung R, Truong D, Conway B. Hepatitis C virus (HCV)-infected people who use drugs (PWUD) with cirrhosis: need for urgent treatment of HCV infection to prevent liver-related complications. Poster presentation. Canadian Liver Meeting. May 24-26, Montreal, Canada, 2019.
301. Thiam A, Chu L, Holeksa J, Magel T, Yung R, Conway B. Homelessness impact on active drug use among hepatitis C infected population. Poster presentation. Canadian Liver Meeting. May 24-26, Montreal, Canada, 2019.
302. Magel T, Holeksa J, Thiam A, Truong D, Conway B. HIV-infected women: medical co-morbidities and rates of virologic suppression. Poster presentation. 10th International AIDS Society Conference. July 21-24, Mexico City, Mexico, 2019.
303. Magel T, Holeksa J, Thiam A, Truong D, Conway B. HIV and sexual health education: an inner-city community based project. Poster presentation. 10th International AIDS Society Conference. July 21-24, Mexico City, Mexico, 2019.
304. Conway B, Smyth D, Thomas R, Wong A, Sebastiani G, Cooper C, Shah H, Béné E, Kumar R, Watson T. Characterization of participants in Canada with chronic HCV infection initiating DAA therapy based on risk for HCV transmission and response to treatment: The real-world C-RESPECT study.

Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.

305. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway B. HCV support groups: do they still have a role in the DAA era? Oral presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
306. Holeksa J, Magel T, Thiam A, Truong D, Yung R, Chu L, Conway B. Strategies to mitigate risk of recurrent viremia among people who inject drugs (PWID) successfully treated for HCV infection. Oral presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
307. Holeksa J, Magel T, Thiam A, Truong D, Ch L, Yung R, Conway B. Low levels of HCV knowledge in key populations: a barrier to HCV elimination. Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
308. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway B. Multidisciplinary care and the four-legged chair: the addiction leg. Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
309. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway B. HCV among cirrhotic people who use/ inject drugs (PWID): response to therapy and long-term follow-up. Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
310. Alimohammadi A, Yamamoto L, Magel T, Wuerth K, Conway B. Real-world efficacy of salvage therapies for the treatment of chronic hepatitis C virus infection in treatment experienced people who inject drugs (PWID). Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
311. Magel T, Wuerth K, Alimohammadi A, Thiam A, Chu L, Yung R, Truong D, Conway B. Morbidity and mortality in HCV-infected people who use drugs (PWUD): beyond the SVR12. Poster presentation. 8th International Conference on Hepatitis Care in Substance Users. September 11-13, Montreal, Canada, 2019.
312. Holeksa J, Magel T, Thiam A, Chu L, Yung R, Truong D, Conway B. Hepatitis C virus (HCV) elimination and the opioid crisis: joint problems, joint solution – results of a pilot program. Oral presentation. Lisbon Addictions. October 23-25, Lisbon, Portugal, 2019.
313. Magel T, Holeksa J, Thiam A, Chu L, Yung R, Truong D, Conway, B. Addressing the opioid crisis: engagement in multidisciplinary care. Poster presentation. Lisbon Addictions. October 24-26, Lisbon, Portugal, 2019.
314. Wuerth K, Magel T, Thiam A, Sian P, Yamamoto L, Truong D, Conway B. Rate of HCV reinfection post-SVR in people who inject drugs (PWID) in a multidisciplinary clinic. Oral presentation. Canadian Society of Addiction Medicine. October 24-27, Halifax, Canada.
315. Thiam A, Sian P, Truong D, Holeksa J, Magel T, Conway B. Substance use patterns among HIV-HCV co-infected individuals post HCV treatment. Oral presentation. Canadian Society of Addiction Medicine. October 24-27, Halifax, Canada.
316. Wuerth K, Magel T, Thiam A, Alimohammadi A, Yung R, Truong D, Conway B. Effectiveness of sofosbuvir/velpatasvir and elbasvir/grazoprevir for the treatment of HCV genotype 1 infection in a real-world cohort with numerous people who use drugs (PWUD). Poster presentation. American Association of the Study of Liver Diseases. November 8-12, Boston, U.S.A, 2019.
317. Alimohammadi A, Yamamoto L, Magel T, Wuerth K, Conway B. Real-world efficacy of salvage therapies for the treatment of chronic hepatitis C virus infection in treatment experienced people who inject drugs (PWID). Poster presentation. American Association of the Study of Liver Diseases. November 8-12, Boston, U.S.A, 2019.
318. Magel T, Wuerth K, Alimohammadi A, Thiam A, Chu L, Yung R, Truong D, Conway B. Morbidity and mortality in HCV-infected people who use drugs (PWUD): beyond the SVR12. Poster Presentation. American Association for the Study of Liver Disease. November 8-12, Boston, U.S.A, 2019.
319. Thiam A, Magel T, Wuerth K, Alimohammadi A, Chu L, Yung R, Truong D, Conway B. Long-term follow-up of people who use drugs (PWUD) following successful HCV therapy: drug use patterns

and maintenance of cure. Poster presentation. American Association of the Study of Liver Diseases. November 8-12, Boston, U.S.A, 2019.

320. Wuerth K, Jones L, Magel T, Yung R, Truong D, Conway B. A new syndemic: opioid misuse and HCV infection. Oral presentation. 21st Annual Meeting of the International Society of Addiction Medicine. November 13-16, New Delhi, India, 2019.
321. Jones L, Magel T, Wuerth K, Yung R, Truong D, Conway B. Engagement in long-term multidisciplinary care: towards elimination of HCV infection and improvement in long-term health. Oral presentation. 21st Annual Meeting of the International Society of Addiction Medicine. November 13-16, New Delhi, India, 2019.
322. Magel T, Wuerth K, Jones L, Chu L, Yung R, Truong D, Conway B. Does engagement in care reduce opioid-related deaths? Oral presentation. 21st Annual Meeting of the International Society of Addiction Medicine. November 13-16, New Delhi, India, 2019.
323. Wuerth K, Magel T, Jones L, Sian P, Yamamoto L, Truong D, Conway B. Towards the elimination of HCV in British Columbia: a strategy to address the "messy middle." Oral presentation. International Viral Hepatitis Elimination. November 22-23, Amsterdam, Netherlands, 2019.
324. Wuerth K, Magel T, Jones L, Sian P, Yamamoto L, Truong D, Conway B. Towards the elimination of HCV in British Columbia: a strategy to address the "messy middle." Poster presentation. International Viral Hepatitis Elimination. November 22-23, Amsterdam, Netherlands, 2019.
325. Conway B, Truong D, Ma B, Sian P, Parsons R, Yamamoto L, Yung R, Jones L, Magel T, Wuerth K. Sofosbuvir/velpatasvir (S/V) for the treatment of chronic HCV in active drug users: the CHIME study. Poster presentation. Canadian Liver Meeting. February 28- March 1, Montreal, Canada, 2020.
326. Magel T, Smyth D, Duncan W, Barrett L, Stewart K, Wong A, Cooper C, Vachon M.L, Borgia S, Ramji A, Macphail G, Fraser C, Hamour A.O, Bullinckx L, Tam E, Feld J, Lee S, Conway B. Efficacy of sofosbuvir/velpatasvir (S/V): impact of treatment adherence. Oral Presentation. Canadian Liver Meeting. February 28- March 1, Montreal, Canada, 2020.
327. Magel T, Wuerth K, Jones L, Chu L, Yung R, Truong D, Conway B. Overdose events among active drug users successfully treated for HCV: the impact of homelessness. Poster presentation. Canadian Liver Meeting. February 28- March 1, Montreal, Canada, 2020.
328. Conway B, Truong D, Rhee D, Yung R, Sian P, Yamamoto L, Parsons R, Jones L, Magel T, Wuerth K. Glecaprevir/pibrentasvir for the treatment of hepatitis C virus infection among active drug users: the GRAND PLAN study. Poster presentation. Canadian Liver Meeting. February 28- March 1, Montreal, Canada, 2020.
329. Jones L, Wuerth K, Magel T, Chu L, Yung R, Truong D, Conway B. Natural history of cirrhotic people who use drugs (PWUD) following successful HCV therapy in the direct acting antiviral (DAA) era. Poster presentation. Canadian Liver Meeting. February 28- March 1, Montreal, Canada, 2020.
330. Magel T, Wuerth K, Jones L, Yung R, Truong D, Conway B. Treatment of HCV infection among people who use drugs (PWUD): a longitudinal real-life cohort. Oral presentation (online). Digestive Disease Week. May 2-5, Chicago, U.S.A.
331. Troung D, Sharma S, Yung R, Conway B, Community based therapy with Sofosbuvir and velpatasvir in the inner city, American Association of the Study of Liver Diseases, virtual, 2021
332. Troung D, Sharma S, Yung R, Conway B, HCV treatment among active inner city drug users with Glecaprevir/Pibrentasvir (G/P): the Grand Plan study, American Association of the Study of Liver Diseases, virtual, 2021
333. Troung D, Sharma S, Yung R, Yi S, Conway B, A simplified approach to antiretroviral therapy with Bikrtarvy among inner city vulnerable residents with HIV infection living up to the promise of 90-90-90, European AIDS conference, virtual, 2021.
334. Troung D, Sharma S, Yung R, Conway B, COVID-19 in the inner city of Vancouver: challenges and opportunities, international society of addiction medicine, virtual, 2021
335. Troung D, Sharma S, Yung R, Yi S, Conway B, COVID-19 in the inner city of Vancouver: challenges and opportunities, international society of addiction medicine, virtual, 2021
336. Troung D, Sharma S, Yung R, Conway B, Prioritizing simplified and decentralized care for marginalized HCV-infected patients: A unique opportunity for addiction medicine, international network of health and hepatitis of substance users, virtual, 2021.

337. Troung D, Sharma S, Yung R, Conway B, HCV elimination: discussion for programs for "birth cohort", indigenous, new-comers, person who use drugs, and incarcerated persons, Global liver forum, virtual, 2021.
338. Troung D, Sharma S, Yung R, Conway B, Long term evaluation of HCV re-infection rates among inner city vulnerable populations, international network of health and hepatitis of substance users, virtual, 2021.
339. Troung D, Sharma S, Yung R, Conway B, Long -term evaluation of HCV reinfection rates among inner city vulnerable populations, American Association of the Study of Liver Diseases, virtual, 2021
340. Troung D, Sharma S, Yung R, YI S, Conway B HCV today: where do we stand with respect to eliminating HCV infection in the inner city, European Association for the study of the liver, London, United Kingdom 2022
341. Troung D, Sharma S, Yung R, Yi S, Conway B, elimination in the inner-city: the road less traveled, Global liver forum, virtual, 2022
342. Troung D, Sharma S, Yung R, Yi S, Conway B, correlates of HCV reinfection among active drug users, international network of health and hepatitis of substance users, international network of health and hepatitis of substance users, Glasgow, Scotland 2022
343. Troung D, Sharma S, Yung R, Yi S, Conway B, HCV treatment among active inner city drug users with Glecaprevir/Pibrentasvir (G/P): the grand plan, international network of health and hepatitis of substance users, international network of health and hepatitis of substance users, Glasgow, Scotland 2022
344. Troung D, Sharma S, Yung R, Yi S, Conway B, Optimizing clinical care in the inner-city populations: innovative community HCV-based clinic, international network of health and hepatitis of substance users, international network of health and hepatitis of substance users, Glasgow, Scotland 2022
345. Troung D, Sharma S, Yung R, Yi S, Conway B, Successful interventions for HCV in the inner city COVID world, international network of health and hepatitis of substance users, Glasgow, Scotland 2022
346. Troung D, Sharma S, Yung R, Yi S, Conway B, the community pop up clinic (CPC): a unique strategy to achieve elimination in the inner city, international network of health and hepatitis of substance users, Glasgow, Scotland 2022
347. Troung D, Sharma S, Yung R, Yi S, Conway B, the community pop up clinic (CPC): a unique strategy to achieve elimination in the inner city, Canadian liver meeting, Ottawa, Canada 2022
348. Troung D, Sharma S, Yung R, Yi S, Conway B, community-based HCV therapy with sofosbuvir/velpatasvir in the inner city, Canadian liver meeting, Ottawa, Canada 2022
349. Troung D, Sharma S, Yung R, Yi S, Conway B, HCV treatment among active inner city drug users with Glecaprevir/Pibrentasvir (G/P): the grand plan, Canadian liver meeting, Ottawa, Canada 2022
350. Troung D, Sharma S, Yung R, Yi S, Conway B, Optimizing clinic care in the inner-city population: Innovative community HCV-based clinic, Canadian liver meeting, Ottawa, Canada 2022
351. Troung D, Sharma S, Yung R, Yi S, Conway B, Successful interventions for HCV in the inner city in COVIDworld, Canadian liver meeting, Ottawa, Canada 2022
352. Troung D, Sharma S, Yung R, Yi S, Conway B, Sofosbuvir/Velpatasvir (S/V) for the treatment of HCV infection among vulnerable inner-city residents: extending the results of the SIMPLIFY study, International viral hepatitis elimination meeting, Amsterdam, Netherlands 2022
353. Troung D, Sharma S, Yung R, Yi S, Conway B, An Innovative Model to ensure access to HCV treatment among vulnerable inner-city residents COVIDworld. American Association of the Study of Liver Diseases, Washington D.C., U.S.A, 2022
354. Troung D, Sharma S, Yung R, Yi S, Conway B, An interim analysis of the CIME project for the provisions of Sofosbuvir/velpatasvir (s/V) for the treatment for HCV infection among inner-city residents with additional vulnerabilities. American Association of the Study of Liver Diseases, Washington D.C., U.S.A, 2022
355. Troung D, Sharma S, Yung R, Yi S, Conway B, Correlates of HCV reinfection among active drug users. American Association of the Study of Liver Diseases, Washington D.C., U.S.A, 2022
356. Yi S, Toniato G, Yung, R, Truong D, Sharma S, Conway B, Sofosbuvir/Velpatasvir(S/V) for the treatment of HCV infection among vulnerable inner-city residents: extending the results of Clinical trial part 2, European Association for the study of the liver, Vienna, Austria, 2023
357. Yi S, Toniato G, Yung, R, Truong D, Sharma S, Conway B, Community pop-up clinic cascade of care

and HCV treatment in Vancouver's inner-city population, European Association for the study of the liver, Vienna, Austria, 2023

(D) **Other**

1. Dawood MR, Allan R, Stackiw W, Conway B, Bechtel LJ, Hirsch MS, Hammond G. Detection of HIV-1 DNA by PCR using structural and regulatory genes on blood samples from seronegative high risk patients [Abstract 1100]. 6th International Conference on AIDS, 1990.
2. Thompson B, Brunham R, Conway B, Loewen R, Duerksen F, Louie T. Diabetic foot osteomyelitis: Risk factors and duration of therapy [Abstract 246]. 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1990.
3. Kradin R, Marathias K, Conway B, Preffer F, Xia W, Pinto C. Pulmonary infiltrating lymphocytes show diminished expression of CD4 antigens. 15th Annual Massachusetts General Hospital Research Symposium, 1991.
4. Rossier E, Lussier M, Miller H, Brodeur B, Conway B, Feibel C. Studies of CMV antigenemia in transplant and AIDS patients. International workshop on CMV, 1991.

5. Denommé N, Fillion D, Fyke K, Pagé S, Clayton D, Bally G, Conway B, Garber G, Cameron DW. Changing mortality: impact of prophylaxis in an AIDS clinic. [Abstract WD.4093]. 7th International Conference on AIDS, 1991.
6. Foisy M, Tierney M, Fillion D, Conway B, Garber G, Cameron DW. Multi-drug therapy in AIDS: prevalence and interactions [Abstract MD.4181]. 7th International Conference on AIDS, 1991.
7. Pon C, Don C, Conway B, Garber G, Walters D, Cameron DW. Sinusitis in HIV infection and immune disease. Abstract MB.2422, 7th International Conference on AIDS, 1991.
8. Daftarian MP, Conway B, Filion LG, Diaz-Mitoma FJ. Anti-sulfamethoxazole antibodies in AIDS patients. Annual Meeting of the Association of Medical Laboratory Immunologists, 1991.
9. Manion DJ, Olberg BJ, Cameron DW, Conway B, Garber GE. Primary visceral Kaposi's sarcoma presenting as ascites. *Clin Invest Med* 14: A78, 1991.
10. Sahai J, Gallicano K, Conway B, Garber G, Foss N, Cameron DW. Correlation of zidovudine pharmacokinetics and response of surrogate parameters of efficacy in HIV. *J Cell Biochem*, 16(Suppl E):82, 1992.
11. Logan DM, Filion L, Conway B, Izaguirre CA. A possible mechanism for the effect of doxorubicin, etoposide, and AZT on epidemic Kaposi's sarcoma. Abstract 17, 2nd Annual Meeting of the Canadian Association for HIV Research, 1992.
12. Sahai J, Gallicano K, Conway B, Foss N, Garber G, McGilveray I, Huang L, Cameron W. Lack of in vivo interaction between naproxen and zidovudine in HIV-infected patients. Abstract presented at the Annual Meeting of the American Society for Clinical Pharmacology, 1992.
13. Pulido-Cejudo G, Ko DS, Gagnon J, Conway B, Izaguirre CA, Campione-Picardo J. Plasma leucine aminopeptidase in HIV-infected patients [Abstract PoA2459]. 8th International Conference on AIDS, 1992.
14. Manion D, Shaw L, Conway B, Diaz-Mitoma F. Prevalence of lymphotrophic Epstein-Barr virus (EBV) variants in immuno-suppressed patients [Abstract 1717]. 32nd Interscience Conference on Antimicrobial Agents & Chemotherapy, 1992.
15. Filion LG, Graziani G, Conway B, Izaguirre CA. Modulation of CD4 expression on primary monocytes. *J Cell Biochem* 17(Suppl E):28, 1993.
16. McFarland EJ, Luckey D, Conway B, Young RK, Ko D, Rosandich ME, Kuritzkes DR. Frequency of HIV-1 gag and envelope-specific cytotoxic lymphocyte precursors in HIV-infected children. *J Cell Biochem* 17(Suppl E):51, 1993.
17. Graziani G, Cormier M, Conway B, Izaguirre CA, Filion LG. CD4 expression by primary monocytes may be upregulated by CSFs. *Can J Infect Dis* 4(Suppl B):13B, 1993.
18. Cameron DW, Hawley-Foss N, Wells G, Victor G, Diaz-Mitoma F, Conway B, Leonard B. Cytomegalovirus retinitis in HIV. *Can J Infect Dis* 4(Suppl B): 23B, 1993.
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This is Exhibit "B" referred to in the Affidavit of Dr. Brian Conway in the City of Vancouver, in the Province of British Columbia before me at the City of Toronto in the Province of Ontario, on March 18, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

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March 15th, 2024,

RE : Fisman et al ats Bridle
Your File No.: 100837

This Medical-Legal Report is written in response to your letter of instruction dated January 19th, 2024, regarding the above-named file. You asked that I express my opinion regarding the science behind the COVID-19 vaccine and the knowledge of medical professionals about the about the vaccine early in the pandemic.

Subsequently, in an email communication of February 27, 2024, you asked that my report address the claims made by the Plaintiff, Dr. Bridle, about the safety of vaccines in an interview in May 2021. In particular, you asked that I comment about:

1. The spike protein issue, including a response to his statement that: "We made a big mistake. We didn't realize it until now, we thought the spike protein was a great target antigen. We never knew the spike protein itself was a toxin and was a pathogenic protein so by vaccinating people, we are inadvertently inoculating them with a toxin"
2. The accumulation in ovaries
3. The risks to children including myocarditis, including his references to published articles and comments from colleagues that share his view.

DUTY TO THE COURT

I am aware that, as an expert, my duty is to assist the court and not to be an advocate for any party. I have prepared my report in conformity with that duty and will abide by that duty if I testify in court.

PROFESSIONAL BACKGROUND AND QUALIFICATIONS

I received my medical degree from McGill University in 1982. After my internship and residency, I completed a fellowship in Infectious Diseases at the University of Manitoba and a fellowship in HIV medicine at Harvard University, ending in 1990. Since that time, I have been in medical practice with a focus on HIV, HCV, and other infectious diseases. With the advent of the COVID pandemic, I have been asked to play a leadership role in informing the public about appropriate public health measures and vaccination practices. I have been interviewed in the lay press over 1,000 times on these subjects. I have been recognized as a reliable source by many media and public health entities. I am the recipient of numerous grants from the public health agency of Canada to study the effects of COVID on the inner city and design appropriate interventions to maximize vaccine uptake. I am currently the president and medical director of the Vancouver Infectious Diseases Centre and an Adjunct Professor in the Faculty of Health Sciences at Simon Fraser University. I work in clinical practice 50% of the time and conduct research 50% of the time. I am widely published in the field of infectious diseases, and I have conducted some of the most important clinical trials in the field, particularly in the field of acute HIV infection, HIV and HCV infection in the inner city and, more recently, COVID. I have published over 150 articles on these and related topics in scientific journals and am asked to teach on these topics on a worldwide basis over the past 25 years or more. In particular, I have been invited to lecture on COVID-related topics on a worldwide basis. My clinical practice includes consultations in the field of general infectious diseases.

A unique aspect of my training and expertise relates to the ability to identify specific concepts in the field of virology and design and/or interpret the studies that must be designed to evaluated and quantify their clinical significance. It is with this specific expertise that I have addressed the questions that have been posed to me.

DISCUSSION OF QUESTIONS

I will begin by addressing his comments about the spike protein, suggesting that administration of the vaccines against COVID (particularly the mRNA vaccines) would lead to the accumulation of large amounts of spike proteins in the body with a wide range of negative effects on a vaccine recipient's well being. There was (and continues to be) a significant medical literature on the possible toxicity of COVID spike proteins to the body (1, 2), so this is, to some extent, correct. However, these effects are associated with natural infection, especially neurologic disease associated with it. As far as the amount of spike protein administered as part of the mRNA vaccines, this is relatively small, almost exclusively limited to the area where the vaccine is administered and surrounding lymph nodes and is broken down by the body within 2 weeks, on average (3). There is no clinical evidence that vaccine-associated spike protein is associated with any clinical disease, in the short and long term. In fact, careful large-scale evaluations of vaccine safety, some published as early as 2021, confirmed the safety of the vaccines (4). This safety has been further confirmed in a study of 100 million vaccinated individuals (5).

For Dr. Bridle to suggest that as a result of the mechanistic possibility of spike protein toxicity and the assumption that, with mRNA vaccines, a large amount of toxic spike protein would be spread throughout the body and cause harm is a theoretical construct that requires clinical validation. This validation has not confirmed Dr. Bridle's concerns in any way. They must be judged to not be of

any clinical significance. Insomuch as his comments makes people decide to avoid vaccination, they need to be informed that being unvaccinated is associated with more severe clinical outcomes of COVID-19 infection: 2.36 times more likely to become infected, 3.37 times more likely to be hospitalized, 6.93 times more likely to be admitted to the ICU (6). One could defend Dr. Bridle's raising these spike protein-related concerns, but his decision to attribute clinical significance to them and to warn people not to be vaccinated on this basis is incorrect and possibly dangerous.

In the same way, his decision to raise the concern about accumulation of mRNA containing lipid nanoparticles begin concentrated in the ovaries and spike protein produced there causing infertility could be defended on scientific ground. Here again, clinical data generated to address this question did not support this hypothesis. It was quickly shown that exposure of ovaries to COVID-19 vaccination does not impair fertility (7, 8). It has further been convincingly demonstrated that COVID-19 vaccination does not affect ovarian reserve in any way (9). Vaccination does not affect spontaneous abortion rates (8). COVID-19 infection in an unvaccinated pregnant woman is associated with a near-doubling in the rate of high blood pressure in pregnancy, pre-term birth and stillbirth (10). Here again, when Dr. Bridle's scientific concerns were tested in real life, they were not found to be of any clinical significance. His decision to attribute clinical significance to them and to warn people not to be vaccinated on this basis is incorrect and possibly dangerous.

His discussion of the issue of myocarditis is based in fact. This side effect is more frequent in younger men and has been confirmed in the recent study that review 100 million vaccine doses (5). It is very fortunate (and Dr. Bridle does not discuss this) that almost all cases that occur can be treated and affected individuals make a full recovery. To use this data as a reason to recommend against COVID vaccination does not present the issue in a fair way. A review of the topic suggests that for each million-vaccine series administered, there will be 10-20 cases of myocarditis, all in individuals under the age of 40. In this series, all recovered (11). For unvaccinated individuals who became infected with COVID-19, the risk of cardiac disease is 40 per million cases. There is a broader range of cardiac disease not seen with vaccination (including pericarditis and cardiac arrhythmias) and a poor outcome in many cases. Dr. Bridle's statement about myocarditis risk is correct, but it must be put in some context. The most appropriate approach is to discuss the risk of vaccination vs. natural infection in the unvaccinated state (particularly in children and adults under 40 years of age) and engage in shared decision making about risk. To simply state that because there is a risk of myocarditis with vaccination that it should simply be withheld for all is, once again, incorrect and possibly dangerous.

In reviewing all the documents shared with me, I would like to raise another issue. This relates to the duration of immunity after vaccine and natural infection. Dr. Bridle has identified the short duration (several months, with a rapid drop off after 4-6 months) of vaccine-induced protection. He also correctly notes that immunity to natural infection lasts somewhat longer. He concludes from this that we should not get vaccinated and simply wait for natural infection to occur. This is an incorrect and possibly dangerous conclusion. First, let us remember the serious consequences of natural infection in the unvaccinated state (6). Extensive studies have shown that the issue of host immunity is nuanced. We now know that if one received 2 vaccine doses in the earlier part of the pandemic, long term protection in about 5%, supporting Dr. Bridle's argument, especially since previous infection without vaccination provides 50% protection (12). If one got 3 vaccines and was not ever infected, protection is actually 60%, and rises to 75% if a previous natural infection

occurs. Thus, it is not that vaccines don't work, it is that their effect wanes over time without proper boosting. If boosting is done, this provides the best long-term protection, especially if natural infection occurred at some point in time, usually causing a more mild disease in the vaccinated state. Vaccines work and save lives if they are used properly, and do not work if used incorrectly. This is the more fulsome explanation of Dr. Bridle's observations, not that vaccines do not work.

Dr. Bridle is a superb virologist and immunologist. His record speaks for itself. In the course of his work, he makes insightful scientific observations, including in the field of COVID-19. However, it is extremely premature to attribute clinical significance to these findings without their rigorous verification in the right setting. In all of the situations I have reviewed above, when Dr. Bridle's observations were tested, their clinical significance was modulated or fully discounted. To use these unverified observations to guide the general public in their decision to receive the COVID-19 vaccine is misleading at best, dangerous at worst.

I trust I have addressed the questions posed to me. If you require any additional information, please do not hesitate to contact me.

Sincerely,

Brian Conway MD, FRCPC
Medical Director
Vancouver Infectious Diseases Centre

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This is Exhibit "C" referred to in the Affidavit of Dr. Brian Conway in the City of Vancouver, in the Province of British Columbia before me at the City of Toronto in the Province of Ontario, on March 18, 2024 in accordance with O.Reg. 431/20, Administering Oath or Declaration Remotely.

Commissioner for Taking Affidavits (or as may be)

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

B E T W E E N:

DR. BYRAM BRIDLE

Plaintiff

and

UNIVERSITY OF GUELPH, JEFFREY WICHTEL, LAURIE ARNOTT, CHARLOTTE YATES, SCOTT WEESE, GLEN PYLE, ANDREW PEREGRINE, DOROTHEE BIENZLE, AMY GREER, DAVID FISMAN, NICK DULEY, JANE OR JOHN DOE JUNIOR SCIENTIST

Defendants

ACKNOWLEDGMENT OF EXPERT'S DUTY

1. My name is Dr. Brian Conway. I live in the City of Vancouver, in the Province of British Columbia.

2. I have been engaged by or on behalf of the Defendant, David Fisman, to provide evidence in relation to the above-noted court proceeding.

3. I acknowledge that it is my duty to provide evidence in relation to this proceeding as follows:

   (a) to provide opinion evidence that is fair, objective and non-partisan;

   (b) to provide opinion evidence that is related only to matters that are within my area of expertise; and

   (c) to provide such additional assistance as the Court may reasonably require, to determine a matter in issue.

4. I acknowledge that the duty referred to above prevails over any obligation which I may owe to any party by whom or on whose behalf I am engaged.

Date MAR/15/24

Signature

NOTE: This form must be attached to any expert report under subrules 53.03(1) or (2) and any opinion evidence provided by an expert witness on a motion or application. RCP-E 53 (July 22, 2014)

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

PROCEEDING COMMENCED AT TORONTO

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RCP-F 4C (September 1, 2020)

DR. BYRAM BRIDLE
Plaintiff

-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

PROCEEDING COMMENCED AT TORONTO

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DR. BYRAM BRIDLE
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-and-

UNIVERSITY OF GUELPH et al.
Defendants

Court File No. CV-22-00691880-0000

ONTARIO
SUPERIOR COURT OF JUSTICE

PROCEEDING COMMENCED AT TORONTO

REPLY MOTION RECORD
OF THE DEFENDANT DAVID FISMAN - Vol 3.
(Returnable November 19, 2024)

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