RaDVaC step-up challenge trial: design and rationale.

Rapid Deployment Vaccine Collaborative (RaDVaC)
Brian Delaney, Preston Estep, Alex Hoekstra, Ranjan Ahuja, Don Wang, and many others

Table of Contents

Licenses

Abstract

We describe the rationale and protocols for establishing rapid and efficient assessment and control of pathogens, through rapidly developed and deployed vaccines. This integrated

framework provides characterization of human immune responses to controlled infection (challenge) with members of a family of closely related pathogens, together with responses to broad-spectrum vaccines against them. Challenge trials (also known as controlled human infection studies) can speed the development of vaccines under most circumstances, and can be conducted safely.[1,2] Here we propose a novel trial structure, called a *step-up challenge trial*, with the following design:

- Challenge an initial study arm[3] with a low-virulence pathogen, measure immune biomarker responses, and establish correlates of protection from disease.
- Vaccinate a second study arm, assess its members for these established correlates of protection, then challenge them with a related higher-virulence pathogen.
- Vaccinate and challenge additional study arms as necessary. For development of vaccines against highly virulent pathogens, additional subdivisions can increase safety.

This step-up model expands the statistical power of typical challenge trials. Such an advance is critically important because typical trials prevent intentional challenge of volunteers who are in groups most vulnerable to pathogens (such as people over age 60), yet immune responses in these volunteers are the most useful in understanding and preventing serious consequences of pandemics. A pan-pathogen vaccine tested and validated in a step-up challenge trial would be ready for rapid deployment upon outbreak of a previously unseen variant in the pathogen family. Moreover, measuring correlates of protection will permit immunobridging approaches to the rapid assessment of new vaccine designs.

Background

Overall RaDVaC project goal (updated 2022-02-19)

The Rapid Deployment Vaccine Collaborative (RaDVaC) was founded by members of the Mind First Foundation in March of 2020 in order to address the outbreak of the SARS-CoV-2 virus, which quickly became a global emergency. The SARS-CoV-2 pandemic produced many notable accomplishments, including the most rapid large-scale vaccine trialing and emergency deployments in history. Nevertheless, vaccines were only available to selected members of the public in high-income countries about a year after initial signs of the emerging pandemic. This

[1] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC7757868/
[2] https://dash.harvard.edu/bitstream/handle/1/42639016/jiaa152.pdf
[3] https://clinicaltrials.gov/ct2/help/arm_group_desc

year-long “vaccine access gap” allowed SARS-CoV-2 to spread largely unchecked throughout the world.

The overall goal of RaDVaC is to bridge or minimize the vaccine access gap–to maximize access to safe and effective vaccines as soon as possible in the event of an outbreak. Currently RaDVaC is achieving this goal by the rapid creation and open-source publication of modular vaccine platforms at the beginning of a serious outbreak of a pathogen, for which no good vaccine(s) are yet approved or available. However, this approach is insufficient for population-scale deployments of regulatory body approved vaccines. Therefore, we herein describe an approach for shortening or shrinking the vaccine access gap for population-scale deployments of approved vaccines. In this approach, broad-spectrum vaccines are pre-trialed, approved for safety, and then can be re-tested rapidly for efficacy upon emergence of a novel pathogen within the target family.

A foundational tenet of RaDVaC is that the best way to accomplish this goal is to share information on vaccine design and testing freely and openly through a global network of researchers engaged in agile R&D. For many researchers, health authorities, and others involved in vaccine development and deployment, such R&D will include not just localized vaccine production but also localized trialing of vaccines.

One of the most important goals of the RaDVaC step-up challenge trial is to establish detailed correlates of immune protection under various conditions of health and vaccination status, as well as genetic background. A correlate of immune protection is an immune marker that has a statistical relation to protection against disease.[4] The establishment of stronger correlates of protection will be critical to enable faster and affordable vaccine trials in geographical regions with resources too limited to conduct large placebo-controlled randomized clinical trials, or expensive basic immunological research. For details about the relevance of correlates of protection to vaccine development, see below, **Immunobridging: Immune profiling and other data analyses**.

While the SARS-CoV-2 pandemic was the catalyst for the initiation of the RaDVaC project, it will not be the last serious outbreak; the establishment of both a robust technical foundation and a distributed scientific network for rapid vaccine deployment are long-term endeavors that will remain just as crucial even as SARS-CoV-2 transitions from pandemic to endemic.

[4] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC3348952/pdf/cis238.pdf

The COVID-19 global emergency (updated 2022-02-15)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; a.k.a. 2019-nCoV; disease: COVID-19) is responsible for a worldwide pandemic far beyond the scope of any other public health crisis in over a century. As of February, 2022, the official worldwide COVID-19 death toll is approaching 6 million. According to analyses by the Institute for Health Metrics and Evaluation, and separately, by Samira Asma, Assistant Director-General of the WHO data and analytics division, the true number of deaths caused by COVID-19 is likely more than three times the official count[5],[6].

As of March 2022, higher-income countries have excellent vaccine access, although substantial fractions of populations in almost all countries refuse to vaccinate. Vaccine access is far worse in low- and middle-income countries. These countries had only minimal access to commercial vaccines through 2021, and will likely not have sufficient access to these vaccines until well after 2022[7]. These significant delays not only lead to continued deaths and chronic illness, but also allow additional viral strains, including escape mutants to arise, compounding the scientific and public health challenges for both low-income and high-income nations, and threatening the fragile steps toward recovery as these new variants spread across the globe.

Because of these large, geographically distributed reservoirs, experts are forecasting that SARS-CoV-2 is transitioning toward being globally endemic. New variants are predicted to emerge regularly for the foreseeable future, causing case and mortality spikes similarly to influenza, though with higher absolute mortality. Only an effective, broad-spectrum coronavirus vaccine has the potential to bring this deadly transition phase to an end. With such vaccines, the unvaccinated might better benefit from next-generation vaccines targeting newer variants, rather than settling for inferior vaccines designed for variants no longer in circulation; and they might also benefit from local testing of these vaccines in the types of trials described herein.

Key advances enabling a new vaccine development platform

Delays in deploying vaccines rapidly and widely are the direct result of the many significant inefficiencies in currently accepted vaccine testing and deployment. One of the most glaring inefficiencies is that, aside from India, the vaccine industry is concentrated far from the places of greatest need: low-income, equatorial and tropical countries. The impact of this mismatch between sites of production and sites of need became clear in the SARS-CoV-2 pandemic:

[5] https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02796-3/fulltext
[6] Samira Asma, WHO press briefing, 2021-05-21. https://www.reuters.com/article/idUSL5N2N81LY
[7] https://www.theguardian.com/society/2021/jan/27/most-poor-nations-will-take-until-2024-to-achieve-[...]-immunisation

through the first quarter of 2021, many countries in need hadn't secured even a single dose of vaccine. Multiple forces have contributed to this dangerous imbalance: 1) the vaccine business has long been unprofitable, forcing many smaller companies to be acquired by larger companies or simply to go out of business, dramatically concentrating vaccine production over the past 30 years[8]; 2) the high cost of R&D and licensing of cutting-edge vaccine technologies, e.g. mRNA; 3) the very high cost of a phase 3 large-scale placebo-controlled randomized clinical trial (RCT), which is typically accepted as the clinical gold standard. But with key advances in critical areas, these historical barriers to vaccine deployment and access could be rendered substantially less hindering.

First, even though the number of vaccine producers has decreased over the past 30 years, many concurrent scientific advancements now allow far more rapid design and preclinical testing of vaccines based on highly safe and efficacious predecessors. These can be produced and adapted quickly, relatively easily, and at low cost. Of particular note are the successes of the BioNTech and Moderna SARS-CoV-2 mRNA vaccines, which were designed, produced and deployed more rapidly than any others in history, and faster than any other competing vaccine platform. Both have also proven to be more effective and more durable than any other vaccine platform, and with a very high safety profile.

Second, the past decade has witnessed the rise of open-source systems and solutions for large-scale critical problems. For example, Linux is the dominant operating system that runs the world's server, cloud, and internet infrastructure, and is used by many businesses; and a majority of cell phones, tablets, and other linked devices run on the Linux-based and open-source Android operating system. The rise to dominance of these open-source operating systems has been driven by demands very similar to those governing vaccine requirements and dependencies--although the call for vaccines is more punctuated by serious and even occasionally desperate local need. Many organizations are starting to recognize the importance of IP-free information sharing, transparency, and interoperability, which allow unprecedented efficiencies and scalability.

Third, advances in immunogenic profiling already provide a number of advantages to vaccine development, including substantial increases in clinical trial efficiencies, stratification and streamlining; more rapid selection of promising vaccine candidates; and substantial reductions in costs, number of participants, and risks. In particular, the potentially very high and occasionally unethical risk posed to trial participants receiving a placebo can be greatly reduced or eliminated

[8] https://www.ncbi.nlm.nih.gov/books/NBK221811/

by augmenting or replacing traditional vaccine randomized controlled trials with vaccine challenge trials.

Taken together, these advances establish a foundation for safe and effective, adaptable vaccine development platforms that are accessible across parameters of geography, economics, technology transfer, and time. These modular platforms can be adapted and tested with the support of public health agencies and the participation of local populations, and further developed through the sharing of data among these agencies across the globe to increase statistical power and to guide improvements in vaccine design, dosing regimens, production, and deployment. As with Linux, for-profit "forks" might play a vital role in increasing vaccine access through large-scale manufacturing.

The importance and ethics of human challenge trials

Controlled human infection studies, or human challenge studies, involve the deliberate exposure of human research subjects to infectious agents.[9] Such trials can result in more rapid deployment of vaccines to those who need them most. Vaccine challenge trials can not only rapidly validate a new vaccine, but can also verify immune correlates of protection standards used to assess protection by vaccines already in use. A recent influenza human challenge trial, for example, showed that the conventional criterion for successful vaccination, a hemagglutination inhibition (HAI) titer of ≥1:40, offered insufficient protection against symptom development, even when viral shedding and duration of illness were significantly reduced.[10]

Challenge trials have already been used widely for many diseases, including the tropical diseases that typically affect many low-income countries.[11] Challenge trials can speed access to effective vaccines where there is an acute need for them. Local production will further increase the efficiency of vaccine deployment by allowing quicker and more effective responsiveness to local outbreaks. Local trialing will also enhance understanding of not only local pathogen variation but also local genetic composition and health concerns, yielding vaccines that are more protective for these particular populations. Indeed, global biosecurity is perhaps best achieved not so much through global, centralized decision making but rather local, decentralized decision making about all aspects of pathogen risk mitigation, including decisions about vaccine design, development, and deployment.

Some professional ethicists have declared their opposition to challenge trials (even small ones), and their preferred support of placebo controlled randomized clinical trials–including trials of tens

[9] For a comprehensive information resource about challenge trials see https://www.1daysooner.org/.
[10] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC4959521/
[11] https://academic.oup.com/aje/article/167/7/775/83777

of thousands, in which many people receiving placebo will become seriously ill and some will die. Other ethicists have challenged this perspective, and suggest that challenge trials are ethically justified, especially in situations where the potential good greatly outweighs possible harm.[12,13,14] In these ethical debates, RaDVaC is concerned with what ultimately is a willingness of some ethicists to permit substantial harm, and an unwillingness to allow or sanction the commission of far less harm.

Our position is simple and clear: *harm permitted is equivalent to harm committed*. Because of multiple factors, the overall level of possible harm in challenge trials is substantially less than the level of harm routinely permitted in placebo-controlled randomized clinical trials. In concordance with our general position, polls of adults have indicated broad support for challenge trials and even a preference for them over placebo-controlled trials.[15] One excellent indicator of the willingness of the public to volunteer for challenge trials is the swelling membership of 1Day Sooner, a non-profit organization that advocates globally for challenge trial volunteers. As of early 2022, over 38,000 people from 166 countries had signed up to volunteer for SARS-CoV-2 challenge trials.

Cost-benefit analysis has shown that, under many conditions, challenge trials save significantly more lives than conventional randomized controlled trials.[16] Moreover, the World Health Organization has made official statements in favor of challenge trials for SARS-CoV-2 specifically, provided that certain guidelines are followed.[17] Nevertheless, and despite the long history of challenge trials in vaccine development, longstanding irrational opposition to challenge trials have rendered them underutilized, and designs have not been optimized for the vetting of multiple modern vaccine platforms.

RaDVaC step-up vaccine trial: rationale

Introduction

General features of effective challenge studies have been published[18], and challenge study guidelines have been described by the World Health Organization.[19] Participant safety must be

[12] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7259898/
[13] https://onlinelibrary.wiley.com/doi/full/10.1002/eahr.500056
[14] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC7476299/
[15] https://www.sciencedirect.com/science/article/pii/S0264410X20315553?via%3Dihub
[16] https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0244418
[17] https://www.who.int/ethics/publications/key-criteria-ethical-acceptability-of-covid-19-human-challenge/en/
[18] https://www.nature.com/articles/s41577-020-00472-0
[19] https://www.who.int/biologicals/expert_committee/Human_challenge_Trials_IK_final.pdf

paramount. Highly informed consent is needed, ideally demonstrated by both a traditional consenting interview as well as a separate enrollment exam[20]. Dosing and preliminary safety data will have been gathered from pre-clinical studies and, if possible, virus characterization and dose ranging studies in humans. When possible, effective rescue therapeutics will be in place.

A typical human vaccine challenge trial consists of the following steps:

- Screening.
- Enrollment.
- Examination and baseline measurements (multiple types of biological samples are typically collected at baseline, and at various points throughout the trial).
- Administration of vaccine or placebo, or even comparator (a different vaccine with known characteristics).
- Challenge with the target pathogen.
- Post-challenge monitoring, measurement of endpoints, and, as needed, treatment.
- Data analysis and interpretation

Conventional challenge trials have a number of advantages over non-challenge, placebo-controlled RCTs: improved knowledge of the disease and transmission; establishment of reliable immune correlates of protection; vastly reduced costs; reduced time to study conclusion; and as a result, more lives saved, especially under pandemic conditions. The dramatically reduced costs and speed of challenge trials is ultimately the result of the large effect size of challenge trials compared to that of RCTs. In a phase 2/3 report of BioNTech/Pfizer's global phase 1/2/3 study of BNT162b2 it took over three months for just 2% of the placebo group to contract COVID-19.[21] With a human challenge study, results can be available in a matter of weeks, and the number of participants can often be well under 100, since the effect size can be 50% or greater. Nevertheless, challenge trials generally are not based on the testing of broad-spectrum vaccines against multiple related human pathogens, and multiple challenge arms of these individual pathogens. Trial designs based on these key factors enable important advances over conventional challenge trials, including the possibility of including participants in groups at higher risk of morbidity and mortality.

We propose a variation on the traditional challenge trial design. One group of study participants receives a broad-spectrum vaccine or placebo, then is challenged–not with the dangerous strain

[20] The online enrollment exam used by the Harvard Personal Genome Project is an example of such a test of sufficient consenting. https://pgp.med.harvard.edu/. Accessed: 2020-03-31.
[21] https://www.nejm.org/doi/full/10.1056/nejmoa2034577
Cited in: https://theactuarymagazine.org/human-challenge-studies/

under investigation (such as SARS-CoV-2), but with a closely related but less virulent strain that typically produces only mild illness (or alternatively, an attenuated version of the dangerous pathogen, created for example by codon deoptimization). Data are collected, analyzed, and a correlate of protection model is derived. A second group of participants receives the same vaccine or placebo, then is challenged with the more pathogenic strain. If participants in high-risk groups (older) are included in the trial, then the COP model can be used to stratify into protected and unprotected, and those predicted to be unprotected (and possibly borderline) will be excluded from the risks of challenge. Ideally, the broad-spectrum vaccine that is used is predicted to be effective against all challenge strains, and the broad activity is due to targeting epitopes that are conserved across all strains. We believe the RaDVaC "step-up" design will generate results more useful than traditional vaccine challenge trials–especially for people in high-risk groups–and with substantially lower risk to the study participants. The step-up design is explained in detail below, in **RaDVaC step-up vaccine trial: design**.

The step-up design has additional advantages for testing modular and multivalent (broad-spectrum, or pan-pathogen family) vaccine platforms, such as those developed and shared by RaDVaC. With previous success in a step-up trial of a vaccine against more than one member of a particular virus group or family (the viruses at each "step"), there would be experimental evidence that prior treatment with the successful broad-spectrum vaccine might provide at least some protection against a new virus in that same family. (This assessment would of course be made in conjunction with virus sequence and structural analysis to determine the areas and degree of overlap in conserved and non-conserved regions.)

If broad-spectrum vaccines are tested and approved preventively, at the beginning of an outbreak of a closely related pathogen (e.g. SARS-CoV-3), the unvaccinated could be given the broad-spectrum vaccine immediately (and already vaccinated people could be given a booster). If a broad-spectrum coronavirus vaccine had been created and trialed for safety and effectiveness prior to the current pandemic, we would have only needed to test effectiveness against SARS-CoV-2. If many had already received the vaccine, those recipients would have pre-existing immunity and partial protection against SARS-CoV-2 that could be reinforced with boosters as needed. Such prevention greatly reduces morbidity and mortality, limiting variant spread and evolution of vaccine resistance.

Slight modifications to the broad-spectrum vaccine might be required to provide robust protection against new pathogen variants, but with a modular vaccine design, such modifications would be achievable without reformatting production infrastructure, and without restarting regulatory approval timelines. Regulatory hurdles should be minimal, since precedents exist in

most jurisdictions for rapid approval of small modifications to already approved vaccine designs, such as annual updates to influenza vaccines. Furthermore, initial vaccination with a broad-spectrum vaccine might overcome potential immune imprinting being reported with development of omicron-specific mRNA vaccines currently in use.[22]

Summary of the rationale for step-up challenge trials

Ultimately, the rationale for the step-up design is based on the following three key points

- Information is needed on high-virulence pathogens, but also on related and common lower-virulence pathogens. Integrated challenge trials for multiple members of pathogen families will provide key details for understanding similarities and differences in immune responses to each pathogen, and it makes sense to trial them in order of ascending virulence, according to a standard protocol.
- The emerging consensus view is that broad-spectrum vaccines for various pathogen groups/families are both possible and highly desirable.
- Correlates of protection derived from low virulence studies potentially will enable higher-virulence challenge of participants in high-risk groups (older or with pre-existing conditions) predicted to be protected by broad-spectrum vaccination. Understanding infection and protection in those at high risk is critically important, and current challenge trial designs avoid controversy by allowing challenge of only low-risk participants, and thus do not meet this need.

Potential scientific and public health benefits of the open-source RaDVaC step-up challenge trial platform

- Can lead to rapid validation and deployment of an experimental vaccine.
- Vastly more affordable than traditional vaccine trials, which require many more participants.
- Iteratively improvable vaccine and trial design.
- Acceleration of advances in immunogenic profiling.
- Can contribute to important scientific questions about COVID-19 (or any pandemic pathogen), including
    - pathogenesis and transmissibility of SARS-CoV-2
    - clinical and molecular immune responses to vaccines and controlled viral challenge, including characterization of antibodies and autoantibodies; and
    - validity of modular vaccine design approaches.

[22] https://www.biorxiv.org/content/10.1101/2022.02.03.479037v1

- Can contribute to important scientific questions about general properties of pathogens, including antigenic cross-reactivity.
- Highly modular – can be used for other pathogens.
- Open-source nature of the platform facilitates sharing among researchers.
- Easy to adapt to local populations and conditions.
- Increased sovereignty: local control and ownership of public health & biosecurity tools.
- Vaccine infrastructure can seed broader public health and biotech efforts around the globe.

Ethical considerations

The greater good

Deliberately infecting a human research subject with a pathogenic virus would appear to run counter to what is often regarded as the most basic tenet of medical ethics: allow no harm to come to the patient. The way this basic tenet is often worded – "do no harm" – highlights an aspect of typical human psychology that makes it difficult for people to see how a human challenge trial can be ethical: people are generally far more concerned with sins of commission than sins of omission or permission.[23] Seeing the net benefit of human challenge trials requires taking into account not just the individual research subjects, but society as a whole. Indeed, several cost-benefit analyses have made it clear that, under most circumstances, human challenge trials are likely to result in significantly more lives saved overall compared to alternative vaccine testing regimens.[24] The reasons are multiple, but the two most important are that human challenge trials 1) can accelerate the development and deployment of vaccines, which begins to save lives sooner than slower deployment would, and 2) can reduce the harm entailed by traditional vaccine trials, which involve sending thousands of trial participants, including those who have taken a placebo, into their communities for many months, where they almost certainly will contribute to the spread of a pandemic pathogen, and where harm will indeed be done to many of them, especially those in the placebo group. Additional reasons include the ability to quickly characterize immune responses to a pathogen, as well as to vaccines, and the ability to gather data on infection and transmission. Once regulators become more comfortable with challenge trials for new pathogens, the time saved – and thus number of lives saved – could be substantial. Berry et al modeled infections and deaths avoided in the U.S. with different types of

[23] https://www.nejm.org/doi/pdf/10.1056/NEJMp1906872
[24] https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0244418

vaccine trials under various conditions involving type of trial and behavior of the population, under the assumption that the trials all would start in August, 2020.[25] See table 1.

Table 1

	Vaccine Efficacy (%)							
	30		50		70		90	
	𝔼[ΔInfections]	𝔼[ΔDeaths]	𝔼[ΔInfections]	𝔼[ΔDeaths]	𝔼[ΔInfections]	𝔼[ΔDeaths]	𝔼[ΔInfections]	𝔼[ΔDeaths]
Status Quo								
RCT	3,914	31	11,539	92	19,130	151	21,557	170
ORCT	5,589	45	16,802	134	33,757	269	50,288	401
ARCT	9,596	76	31,473	250	66,641	531	83,522	665
HCT (30-day set-up)	140,731	1,124	152,263	1,216	156,885	1,254	159,876	1,277
HCT (60-day set-up)	110,046	879	118,937	950	122,482	979	124,777	997
HCT (90-day set-up)	86,466	690	93,370	745	96,111	768	97,886	782
HCT (120-day set-up)	68,213	544	73,611	587	75,747	605	77,132	615
Behavioral								
RCT	363,382	2,845	386,081	3,026	397,396	3,117	404,562	3,174
ORCT	1,139,585	9,061	1,377,157	10,955	1,426,014	11,345	1,457,500	11,598
ARCT	2,588,881	20,647	3,248,449	25,924	3,389,541	27,052	3,473,035	27,720
HCT (30-day set-up)	3,903,566	31,167	4,309,316	34,411	4,481,448	35,789	4,591,750	36,671
HCT (60-day set-up)	2,795,316	22,301	3,082,676	24,598	3,205,159	25,579	3,283,975	26,209
HCT (90-day set-up)	2,011,244	16,028	2,211,985	17,633	2,297,350	18,316	2,352,436	18,757
HCT (120-day set-up)	1,466,239	11,668	1,605,833	12,784	1,664,613	13,255	1,702,601	13,558
Ramp								
RCT	1,075,634	8,316	1,131,531	8,764	1,160,564	8,996	1,179,234	9,145
ORCT	2,853,202	22,569	3,839,945	30,432	3,973,769	31,501	4,050,013	32,111
ARCT	5,711,310	45,401	7,442,922	59,253	7,924,650	63,107	8,071,866	64,285
HCT (30-day set-up)	8,744,377	69,672	9,452,413	75,330	9,725,022	77,511	9,897,591	78,892
HCT (60-day set-up)	6,814,762	54,235	7,381,425	58,762	7,602,878	60,534	7,743,514	61,659
HCT (90-day set-up)	5,266,925	41,851	5,711,663	45,404	5,887,421	46,811	5,999,381	47,706
HCT (120-day set-up)	4,053,134	32,141	4,396,033	34,879	4,532,400	35,970	4,619,521	36,667

https://doi.org/10.1371/journal.pone.0244418.t002

Infections and deaths avoided with vaccine trials.

Status quo: stay-at-home orders and other infection control measures remain in place until the end of the pandemic; *behavioral*: volunteer reduction of social contact with perceived danger, increase in contact with perceived reduction in danger; *ramp*: partial reopening with strict monitoring, limited quarantines as needed. *RCT*: randomized clinical trial; *ORCT*: RCT with optimized surveillance period; *ARCT*: adaptive vaccine efficacy RCT; *HCT*: human challenge trial.

Safety of the individual

While the perspective of the greater societal good is essential for rational decision making about vaccine research regimens, the standpoint of the safety of the research participants is still

[25] Ibid.

paramount in trial design. Human challenge trials have been used to test vaccines for over two hundred years, and many of these studies would be considered unethical, in some cases horrifically so, by modern standards. In order to minimize the potential risks and harm of early challenge trials, general standards have been developed to ensure the safety of participants in challenge trials,[26] and the World Health Organization has recently issued ethical guidelines specific to human challenge trials with SARS-CoV-2,[27] though these guidelines apply to challenge trials with any dangerous pathogen.

WHO's criteria for the ethical acceptability of COVID-19 human challenge studies are summarized in table 2.

Table 2. Eight criteria for SARS-CoV-2 challenge studies

Scientific and ethical assessments		
Criterion 1	Scientific justification	Specific challenge studies must have strong scientific justification
Criterion 2	Assessment of risks and potential benefits	It must be reasonable to expect that the potential benefits of SARS-CoV-2 challenge studies outweigh risks
Consultation and coordination		
Criterion 3	Consultation and engagement	SARS-CoV-2 challenge research programs should be informed by consultation and engagement with the public as well as relevant experts and policy-makers
Criterion 4	Coordination	SARS-CoV-2 challenge study research programs should involve close coordination between researchers, funders, policy-makers and

[26] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4586124/
[27] https://www.who.int/ethics/publications/key-criteria-ethical-acceptability-of-covid-19-human-challenge/en/

		regulators
Selection criteria		
Criterion 5	Site selection	SARS-CoV-2 challenge studies should be situated where the research can be conducted to the highest scientific, clinical and ethical standards
Criterion 6	Participant selection	SARS-CoV-2 challenge study researchers should ensure that participant selection criteria limit and minimize risk
Review and consent		
Criterion 7	Expert review	SARS-CoV-2 challenge studies should be reviewed by a specialized independent committee
Criterion 8	Informed consent	SARS-CoV-2 challenge studies must involve rigorous informed consent

These guidelines are thorough and can serve as the starting point for a local implementation of any challenge trial design. But scientists, physicians, regulators, and other interested parties in any given region will need to adapt these guidelines to local conditions. For example, factors such as the general health of the local population, the state of the pandemic, availability of the resources needed to manufacture and store challenge strains, and so on, may impact the challenge trial design or even the decision to conduct such a trial at all. Ideally, however, research groups working on the same vaccine at different locations should seek to harmonize as many aspects of trial design as possible in order more easily to pool data, which otherwise, at the level of an individual trial, may be underpowered to examine certain potentially valuable secondary and exploratory endpoints.

Site selection (Criterion 5), is important for a number of reasons, one of which involves the ability to ensure the safety of not just the participants, but also the research staff. Adequate measures, including the provision of needed personal protective equipment, must be guaranteed.

One problem posed by any challenge trial with a novel virus such as SARS-CoV-2 results from a lack of long-term data on the consequences of infection. This makes the needed rigorous informed consent particularly complicated, since the long-term risks to which potential participants would be consenting are mostly speculative. This lack of data on risks of exposure to any new or relatively unstudied pathogen must be made clear to study volunteers.

Participant selection (WHO Criterion 6) is extremely important, in order to minimize potentially significant long-term risk. Unfortunately, there still exists only a relatively coarse understanding of risk categories for life-threatening cases of COVID-19, and our understanding of the risk factors for long COVID is weaker still. More precise correlates of protection against disease are needed to completely prevent such outcomes. The RaDVaC step-up design can facilitate identification and contextualization of such correlates in a manner that is safer than traditional challenge studies.

Unique ethical safeguard of the RaDVaC step-up design

Many viral families, including coronaviruses, have well-conserved genome organization and proteomic functions. Across the 7 coronaviruses that infect human hosts (HCoVs), there are some minor differences in sequence and proteome composition, but all major proteins play the same roles, and all family members infect host cells of the respiratory tract in a similar manner[28]. SARS-CoV, SARS-CoV-2, and HCoV-NL63 primarily use the ACE2 receptor, while the other four common coronaviruses use other or unknown receptors. Infection by any of the 7 HCoVs occurs through similar mechanisms, with host proteolytic cleavage of the outer Spike protein resulting in a dramatic rearrangement of the structure of Spike, driving fusion with the host cell. Viral replication, general cell tropism, and interactions with innate immune receptors and immune cells display many similarities; although, differences in virulence result from differences between each pathogen, including differences in host cellular tropisms,and functions of non-conserved accessory proteins. Due to these factors, the more highly virulent coronaviruses replicate very rapidly, infect more cell types, and obviously cause more extreme host immune

[28] All except for MERS infect upper respiratory tract cells in human hosts; MERS infects upper respiratory tract cells in camels but primarily lower respiratory tract cells in humans

responses.[29,30,31,32,33] Nevertheless, because of extensive sequence, structural, and functional similarities, it is expected that the less virulent members of coronaviruses will elicit similar but attenuated immune responses relative to more virulent members.

These conserved features enable the design of broad-spectrum vaccines and step-up challenge trials for testing these vaccines. By first challenging participants, after vaccination, with a well-characterized and relatively benign coronavirus (e.g. NL63), or an attenuated virus, and correlating measured post-vaccination immune response with the development of symptoms and viral shedding, models can be generated and used to predict likelihood of protection against that particular coronavirus or attenuated strain. Given the likelihood that the ability to mount an effective immune response to one type of coronavirus would likely mean at least some ability to mount an effective immune response to other coronaviruses (though this is of course part of what the step-up trial will investigate), we can expect that risk can be reduced substantially by selecting, for the next group in step 2 – to be challenged with SARS-CoV-2 – only those who have the profile that was most protective in the first challenge.

Study objectives

Each study center will have different objectives, and local regulations will constrain the specific study objectives chosen, as well as how, or whether multi-phase trials – such as phase 1/2, or even phase 1/2/3 trials – will be possible. The following represents just one of many possible ways of structuring study objectives, under the assumption that a multi-phase trial structure is feasible and regulatorily permissible. Classification of non-phase 1 goals – secondary and exploratory – is partly arbitrary, and partly dependent on resource availability and local regulatory guidelines.

Ideally, an adaptive trial design would be used, since adaptive trials are best suited to dose discovery studies and other early phase trials[34] – especially studies with an experimental design. Adaptive trial designs also make sense in emergency pandemic situations, where the extent of the health emergency often changes rapidly, and therewith the need for flexibility in order to deploy solutions without undue delay.

[29] https://pubmed.ncbi.nlm.nih.gov/35079775/
[30] https://pubmed.ncbi.nlm.nih.gov/34242356/
[31] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7641391/
[32] https://onlinelibrary.wiley.com/doi/full/10.1111/resp.13196
[33] https://onlinelibrary.wiley.com/doi/full/10.1002/path.4454
[34] https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-018-1017-7

The merits of the step-up trial design are independent of the pathogen being investigated. However, the motivation for the development of the RaDVaC step-up challenge trial is the ongoing COVID-19 pandemic. For this reason, in what follows we will largely outline the general principles of the step-up design in terms of the SARS-CoV-2 virus, and, more specifically, in terms of the RaDVaC RNA pan-corona virus vaccine, PanCoV214.

Primary objective

The primary objectives of the RaDVaC step-up challenge trial are to:

- Measure the safety and tolerability (reactogenicity) of RaDVaC's PanCoV214 (and ideally other SARS-CoV-2 vaccines)
- Measure the safety and tolerability of 2 separate doses, given to separate study arms, of an attenuated strain of SARS-CoV-2, or possibly a relatively benign common coronavirus such as HCoV-OC43, or the somewhat more virulent and less common HCoV-NL63
- Measure the safety and tolerability of 2 separate doses, given to separate study arms, of SARS-CoV-2

Secondary objectives

The secondary objectives of the RaDVaC step-up PanCoV214 challenge trial are to

- Measure deep immune profile changes after inoculation with PanCoV214
- Observe symptoms of disease and establish immune and other correlates of protection based on 2 separate doses, given to separate study arms, of a lower-risk virus
- Observe symptoms of disease and establish immune and other correlates of protection based on 2 separate doses, given to separate study arms, of SARS-CoV-2
- Gather initial data on the efficacy of the vaccine against both the lower-risk virus and SARS-CoV-2

Exploratory objectives

- Explore which elements of measured molecular signatures and other aspects of immune and health profiles seen in effective responses to the benign viral challenge are also correlated with effective responses to the challenge with the more pathogenic virus
- Examine degree of cross-reactivity of antigens to different viruses within a family (or other taxa)
- Refine broad-spectrum vaccine designs
- Begin to validate the step-up trial model

- Field-test an open-source platform for global pooling of data from trials using systems biology to pursue scientific goals such as establishing correlates of protection by vaccines under varying circumstances and in different populations

RaDVaC step-up vaccine trial: design template and principles

Introduction

Two separate advances come together to make the RaDVaC step-up trial design possible. One is the modular design of the RaDVaC SARS-CoV-2 vaccine platforms, which permit relatively broad-spectrum design. The other is the step-up trial design itself.

The potential value of a broad-spectrum or pan-coronavirus vaccine has been recognized by many scientists and public health officials.[35] RaDVaC has designed an open-source pan-coronavirus vaccine (PanCoV214) by adapting the principles of our SARS-CoV-2-specific vaccine design to an RNA-based vaccine platform. The proposed PanCoV214 vaccine contains highly conserved pan-coronavirus family epitopes, mined partly from studies showing antibody cross-reactivity.

Purpose of the step-up trial design template

The step-up trial design is, to the best of our knowledge, entirely novel; the trial design itself should thus be fine-tuned in an experimental way, by testing variations in design parameters.

Given the value of many different types of implementation of the step-up design principles, we present in what follows only a general outline of one possible implementation of the overall RaDVaC step-up trial design. Details in the example design that follows, such as study arm size and randomization strategy, are merely suggestive. Statistical and sensitivity analyses will need to be performed to establish a concrete data plan.

In addition, other trial details will be determined by local authorities and researchers. A full study protocol, with all the important components required by health agencies and ethics boards, can be developed from the outline below based on the needs and regulations of a given region or jurisdiction.

[35] https://pubmed.ncbi.nlm.nih.gov/33315546/

Step-up trial design

The step-up trial design has several elements that go beyond a traditional challenge trial. The step-up design involves the following steps:

- Screening.
- Enrollment.
- Baseline immune-profiling.[36]
- Examination, and administration of vaccine to Step 1 group.
- Deep immune-profiling again ≃ two weeks after final booster.
- Examination, and administration of vaccine to potential members of Step 2 group (around three weeks after Step 1 group receives their first vaccine treatment).
- Challenge in Step 1 group with the milder pathogen.
- Post-challenge monitoring, measurement of endpoints, and, as needed, treatment.
- Establishment of correlates of protection.
- Selection, from potential members of Step 2 group, of those with optimum immune profiles based on Step 1 group responses. With the availability of effective rescue therapeutics, it would probably be safe to challenge all participants in low-risk groups, including those in the placebo group.
- Challenge in Step 2 group with higher-virulence pathogen.
- Post-challenge monitoring, measurement of endpoints, and, as needed, treatment.
- Refinement of initially established (in Step 1) correlates of protection.
- (Multiple types of biological samples can be collected at key points throughout the trial.)

Many variations on this design are possible, and perhaps even required in certain locales under certain pandemic conditions. The global research community will in fact benefit from the field-testing of multiple variations of this overall design. We encourage other scientists to adapt this as needed or desired not only for SARS-CoV-2 trials, but also for studies seeking to validate vaccines against other pathogens.

Facilities

The choice of study site and facilities will need to be made by local researchers and medical authorities. Ideally, any challenge trial with a pathogen capable of causing serious disease would be conducted in an isolation unit or other controlled environment. In many cases, however, this will not be practical, and might not be considered necessary by local health authorities, especially

[36] See section below: Deep Immune Profiling

in the case of a study using a mild pathogen. The risk to the study must also be taken into account: a research participant could herself be infected by a pathogen in the community, which would skew the results.

Study population

Infectious challenge trials currently being considered for SARS-CoV-2 are intending to recruit young, healthy participants, usually ages 18–30,[37] for the simple reason that COVID-19 is much less likely to cause serious disease in the young (assuming they don't have other risk factors for severe COVID-19). One of the key advantages of the step-up design is that immune profiling of the group of volunteers in the first step will yield immune correlates of protection that could reduce risk in the second step (the challenge with SARS-CoV-2). Depending on local availability of rescue therapies at the time the trial is conducted, this could mean that the upper age limit for entry into the trial could safely be increased, perhaps substantially. This might help solve a problem that has vexed much research into COVID-19: the heavy reliance on young research subjects, which leaves open the question of whether study results are applicable to older people, who of course are the most vulnerable to severe COVID-19 disease.

Our intended study population is hence healthy adults, ages 18 to 55. In the design implementation that follows, the total number of participants to be recruited is 100 (for the study of one vaccine; additional participants are required for each additional vaccine). The total number of participants needed, as well as study arm sizes, may be higher, and can be more precisely determined by trial simulation and statistical analyses. Further, as with many other elements of a developed protocol, local conditions will be determinative of ideal trial size. For example, attrition rates may vary substantially from region to region, which may require a larger study arm. See below, **Statistical powering, randomization, blinding, and group assignment**, for more details.

Screening

Pre-existing immunity is a serious but often ignored confounding factor in clinical trials of vaccines, and is even more important in a step-up trial design.[38] First, volunteers will be prescreened by questionnaire for prior vaccination to SARS-CoV-2. These volunteers can be placed into a pre-vaccinated arm (to study various phenomena, including the immunogenic effects of updated boosters). Second, all volunteers will be prescreened biochemically for prior exposure both to SARS-CoV-2, and – if the choice is made to use a benign coronavirus in step 1

[37] https://www.hra.nhs.uk/planning-and-improving-research[...]/sars-cov-2-characterisation-study-covid-19/
[38] https://www.mdpi.com/2076-393X/8/4/683

of the viral challenge, as opposed to an attenuated version of SARS-CoV-2 – to whatever virus is chosen. (There are many options for a viral agent in step 1 of the challenge. See below, **Choice of virus for step 1 challenge**.)

Next, a study physician will examine the volunteer and make a decision about his or her entry into the trial based on the inclusion and exclusion criteria.

Each research group will determine its own inclusion and exclusion criteria. Most clinical research inclusion and exclusion criteria are common to all medical research studies seeking healthy human research subjects. But several exclusion criteria to be used in the RaDVaC PanCoV step-up challenge trial are worth noting, since they will be essential in this design involving a vaccine against a respiratory illness.

Exclusion criteria specific to the RaDVaC step-up challenge trial with vaccine

- (From prescreening.) Serological antibody evidence of prior exposure to SARS-CoV-2 or any more benign pathogen used in Step 1.
- Smoker. Participants must never have smoked, or stopped six months before trial start.
- Presence of significant acute or chronic respiratory illness or lung disease of any kind.
- (If intranasal delivery.) History of hay fever or significant allergic rhinitis.
- (If intranasal delivery.) Current use of any intranasal medication.
- Receipt of any vaccine within 4 weeks prior to trial start.
- History of frequent nose bleeds.
- Any nasal or sinus abnormality that could affect 1) delivery of the viral challenge, 2) delivery of the vaccine, or 3) collection of needed samples (for example via nasal washing).

Informed consent and enrollment

Informed consent must be rigorous. We envision a three-step process. First, prospective research participants will read material, and/or watch a video about the study and attendant risks. This material will be provided online. Next, the volunteers will take an online exam to ensure that they have understood the trial and the risks their participation will entail. Consenting via an online exam with no live researcher oversight is an accepted, and – especially during a pandemic caused by a highly transmissible pathogen – attractive means of ensuring that volunteers understand the risks of a study they are considering joining.[39] Finally, as an extra safeguard, a

[39] This type of online, "researcher-free"/automatic consenting was pioneered by the founders of the Harvard Personal Genome Project: https://pgp.med.harvard.edu/

researcher or legally authorized representative will conduct a more traditional face-to-face consent process – though possibly via video – to be certain the volunteer understands the risks of participating in the study. (All online material, and the exam itself can also be made available in printed form, or on computers at a central community location.)

Randomization, blinding, group assignment, and sample size

Fully blinded, simple randomization (the equivalent of a truly random coin toss for each group assignment decision) is the most effective way to prevent various forms of bias – selection bias resulting in non-random distribution of participants with the same covariates into the same arm, effects of knowledge of group assignment on assessments of results, and so on.

One downside to the use of simple (or full) randomization is that it requires a large number of participants in order to reduce the risk of an excessively lopsided distribution of people into treatment arms. Statistical methods can of course take into account such lopsided distributions, but with small study sizes, there is a significant chance that full randomization will result in participant distributions that make it difficult to produce meaningful research results. For a step-up challenge trial testing one vaccine, the number of research arms is large enough that full randomization is probably unwise with a total participant pool of less than 150 or so. Yet many research groups don't have the resources to conduct a study that large. Moreover, in certain locations, recruitment of such a large number of people may take significantly longer than recruiting a smaller number of participants, and the resulting delays in trial execution could cost lives during a pandemic.

Block randomization is often used in small studies to ensure equal distribution across study arms, and can remove sources of bias as effectively as full randomization, although covariates must be controlled for carefully.[40] For this reason, we believe a total initial participant pool of just 100 people may be acceptable, but trial modeling should be conducted to confirm this. Because of the fixed block sizes, full double-blinding will not be possible (the block sizes themselves will be apparent). Moreover, the small block size means treatment allocation might be ascertainable. If these limitations – which are relatively minor – are unacceptable, larger numbers of participants can be recruited.

Participants will be assigned via block randomization to one the following three groups. (Groups 1 and 2 will later be split in half, each to receive different challenge doses, low or high.)

Many studies have adopted this style of consenting, even before the current pandemic made it a much more compelling choice. One example is the US NIH's "All of Us" research study: https://allofus.nih.gov/

[40] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3136079/

1. (Step 1 group) vaccine, lower-risk challenge (20 participants).
2. (Step 1 group) placebo, lower-risk challenge (20 participants).
3. (Step 2 group) participants to be challenged with low- and high-dose SARS-CoV-2, to be randomized separately later (60 participants in the initial pre-selection pool).

We expect that the immune correlates of protection revealed by the first step of the study will disqualify a significant number of the participants slated to receive the SARS-CoV-2 challenge, hence the uneven initial division of participants between the lower-risk challenge group (step 1), and the rest – some, but very likely not all of whom will be in the SARS-CoV-2 challenge group (step 2).

Determining the correct sample size for any clinical trial is an important and often challenging task.[41] Because the effect size of a challenge trial is high, statistical significance is easily achieved with small sample sizes. Most human challenge trials involve challenging just 30-60 people, which is sufficient to achieve statistical significance.[42] The first SARS-CoV-2 challenge trial to be conducted challenged 34 people.[43] The RaDVaC step-up challenge trial, however, has three features that will require a greater number of participants. First, each of the two steps to some degree constitutes a separate arm, which means the total number of participants needs to be doubled. Second, it is very difficult to estimate how many people initially enrolled and reserved for step 2 will have an immune response to vaccination (or placebo) that will enable them to be safely challenged. Finally, a central goal of the study is exploratory: we seek to identify correlates of protection involving not just how the vaccine produces changes in the immune system, but how the induced changes interact with other variables, such as HLA type. These other variables are numerous enough that a study size of thousands could potentially yield unique discoveries not possible with a study size of one or two hundred. But the statistical concern with powering a study sufficiently needs to be balanced by the ethical concern about minimizing the number of people exposed to a health risk.[44] Taking these factors into account, we have chosen to illustrate the step-up challenge trial with an initial enrollment size of 100 people, which should strike the right balance between the power to test vaccines and discover new correlates of immunity, on the one hand, and avoidance of needless risk to participants. But trial simulation or modeling should be used in developing a complete protocol in order to verify choice of sample size.

[41] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC7745163/
[42] See, for example:
https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC6880340/
https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC4959521/
https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC7353841/
https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC3732056/
[43] https://www.researchsquare.com/article/rs-1121993/v1
[44] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC3148614/

Optional: preliminary dose-finding studies

Vaccine dose

The dose of vaccine used can be guided by other vaccine studies and experience with RaDVaC's previous SARS-CoV-2 vaccine designs. See below, **Dosing and booster schedules**.

Viral dose

To achieve sufficient statistical power with a minimum of research subjects, a viral dose should be used that is sufficient to cause disease in a majority of those exposed, but not cause disease that is more serious than is typical for natural infection[45]. Several challenge trials with low virulence human coronaviruses have been conducted,[46,47] which can help guide dosing for Step 1, and, as of August, 2021, one challenge study with SARS-CoV-2 is ongoing,[48] and others will no doubt follow. In addition, numerous non-human primate challenge trials have been conducted with coronaviruses,[49,50,51,52] although the suitability of various non-human primate species for modeling appropriate viral challenge dosing in humans is still yet to be determined.[53]

Step 1 study arm

Step 1 group, day 1. Study visit #1: exam and first vaccine or placebo treatment

During the first study visit, the participants in the lower-risk challenge group will be examined by medical staff to 1) rule out pregnancy or any other disqualifying conditions that might have arisen since the enrollment exam, 2) take baseline clinical measurements, and 3) take samples for deep immune profiling.

Next, half of the step 1 group (20) will be inoculated with PanCoV214, and the other half (20) with a placebo. Participants will be monitored closely for acute reactions, treated as needed, then moved to the in-patient facility where they will remain for the first 45 days of the study.

Participants will continue to be monitored and instructed to report any unexpected symptoms.

[45] https://academic.oup.com/jid/article/221/11/1752/5814216
[46] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1843247/
[47] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7110151/
[48] https://www.hra.nhs.uk/planning-and-improving-resea[...]sars-cov-2-characterisation-study-covid-19/
[49] https://www.nature.com/articles/s41422-020-0364-z
[50] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7706928/
[51] https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0246366
[52] https://www.biorxiv.org/content/10.1101/2020.02.17.951939v1.full
[53] https://www.biorxiv.org/content/10.1101/2020.04.08.031807v2

Step 1 group, day 11 and day 21. Study visits #2 and #3: boosters (or placebo)

After ten days, participants in the lower-risk challenge group will receive their second treatment with either PanCoV214 or the placebo (depending on the arm they're in), and, as before, will be monitored for acute reactions, then will return to the in-patient facility. This will be repeated for the third treatment after a total of 20 days from the start of the trial.

Step 1 group, day 31. Study visit #4: clinical and immune assessment and viral challenge

After a total of 30 days from trial start, all 40 participants will return to the study center for 1) final clinical examination and sample collection for deep immune profiling and 2) challenge via nasal drops with the lower-risk virus.

The 20 participants in each of the two groups, placebo and vaccine, will be block-randomized into two groups of five each, and treated as follows.

- placebo, low-dose challenge (10 participants)
- placebo, high-dose challenge (10 participants)
- vaccine, low-dose challenge (10 participants)
- vaccine, high-dose challenge (10 participants)

See below, "Dosing and Administration", under **Step 1 challenge virus**, for suggested viral challenge doses.

After being checked for acute reactions, the participants will return to the in-patient housing facility where they will be monitored closely for 14 days. Treatment of any symptoms that develop will be provided as needed.

Step 1 group, day 45. Establish participant selection criteria for SARS-CoV-2 (step 2) challenge

The primary criteria for selection from the second group of participants for inclusion in the group to be challenged in step 2 are signs in this group of an immune response to the vaccine that matches those seen in step 1 participants who were most protected from disease by the vaccine.

Machine learning will be used to identify the most salient elements of the immune response to the vaccine in those from step 1 who mounted the strongest response to the vaccine. Those participants not yet challenged will be selected based on similarity of vaccine response. (For details, see below, **Immunobridging: Immune profiling and other data analyses**.)

Participants from step 1 will be able to leave the in-patient facility at this time.

Step 2 study arm

Step 2 group, day 1*. Study visit #1: selection, randomization, physical examination, and first vaccine or placebo treatment

** Note, in order to produce results as quickly as possible, the step 2 group could begin receiving vaccine (or placebo) earlier, on (overall) study day 20 or so, assuming that full vaccination takes around 30 days, and that deep immune profiling results will be available around 5 days after taking samples.*

The 60 participants in the step 2 group will be examined by medical staff as before with the step 1 group, then block-randomized into placebo group and treatment group, with 30 participants in each.

The treatment group will be inoculated with PanCoV214, the placebo group with the placebo. Participants will be monitored closely for acute reactions, treated as needed, then moved to the in-patient facility where they will remain for the first four weeks of the study.

Participants will continue to be monitored and instructed to report any unexpected symptoms.

Step 2 group, day 11 and day 21. Study visits #2 and #3: boosters (or placebo)

After ten days, participants in the SARS-CoV-2 challenge group will receive their second treatment with either PanCoV214 or the placebo, and as before will be monitored for acute reactions, then will return to the in-patient facility. This will be repeated for the third treatment after a total of 20 days from the start of step 2 group inoculation.

Step 2 group, day 31. Study visit #4: clinical and immune assessment

After a total of 30 days from the start of step 2 inoculation, all 60 (minus any withdrawals) step 2 participants will return to the study center for final clinical examination and sample collection for deep immune profiling. Participants will then return to the in-patient facility where they will wait for the selection process to take place for the next phase of the study.

Step 2 group, day ≈36. Study visit #5: viral challenge

When 1) the results of the deep immune profiling are complete, 2) profile-based selection criteria for the challenge phase established, and 3) the selection of participants able to proceed safely to the challenge phase of the trial is made, participants able to proceed to the next phase of the trial

will return to the study center for final clinical examination and challenge with SARS-CoV-2 via nasal drops.

Note: It is of course highly unlikely – though by no means impossible – that those in the placebo group will have developed correlates of protection matching profiles seen in effective vaccination in step 1 (though they may have had them from the beginning). The health of the placebo group participants, and, above all, availability of rescue therapies, can be used by the researchers to determine the suitability of the non-vaccinated participants to receive a challenge with SARS-CoV-2. Block randomization will again be used to assign participants to groups as follows:

- Placebo, low-dose challenge (half of placebo group, likely ≈0–2 participants); skip if deemed too dangerous
- Placebo, high-dose challenge (half of placebo group, likely ≈0–2 participants); skip if deemed too dangerous
- Vaccine, low-dose challenge (half of vaccinated group)
- Vaccine, high-dose challenge (half of vaccinated group)

See below, “Dosing and Administration”, under **Step 2 challenge virus**, for suggested viral challenge doses.

After being monitored for acute reactions to the challenge, the participants will return to the in-patient housing facility where they will be observed closely for 14 days. Appropriate treatment will be provided as needed if any participants present with COVID-19 symptoms.

Step 2 group, day ≈50. Study visit #6: clinical assessment

All participants will be assessed clinically one more time for signs of COVID-19 disease. Samples will be taken for deep immune profiling to establish immune correlates of protection. Participants who are ill with COVID-19 will continue to receive treatment as needed. The decision of where to continue to care for these people – at the in-patient facility, at home, or elsewhere – will be made by the local study physician(s).

Follow-up

Participants will be given contact information for the principal investigator(s) and instructed to report any symptoms they feel could be related to their participation in the trial.

Adverse event reporting

Monitoring for and reporting adverse events (AEs) is always an essential part of any clinical trial. With an open-source trial design, modified and used by researchers in diverse contexts sharing data with one another, rapid adverse event reporting becomes critical. Aside from following the relevant requirements of the regulatory body overseeing the particular trial, researchers should log, at a minimum:

- A description of the AE
- Whether it was a serious adverse event
- An assessment of the likelihood of a causal relation to treatment (the vaccine, or the challenge)

More detailed suggestions can be found in the template protocol developed by the Brighton Collaboration.[54]

An online platform will exist for researchers to share adverse event information.

Individual components of the trial: further details and rationales

Selection of vaccine(s) for trial inclusion

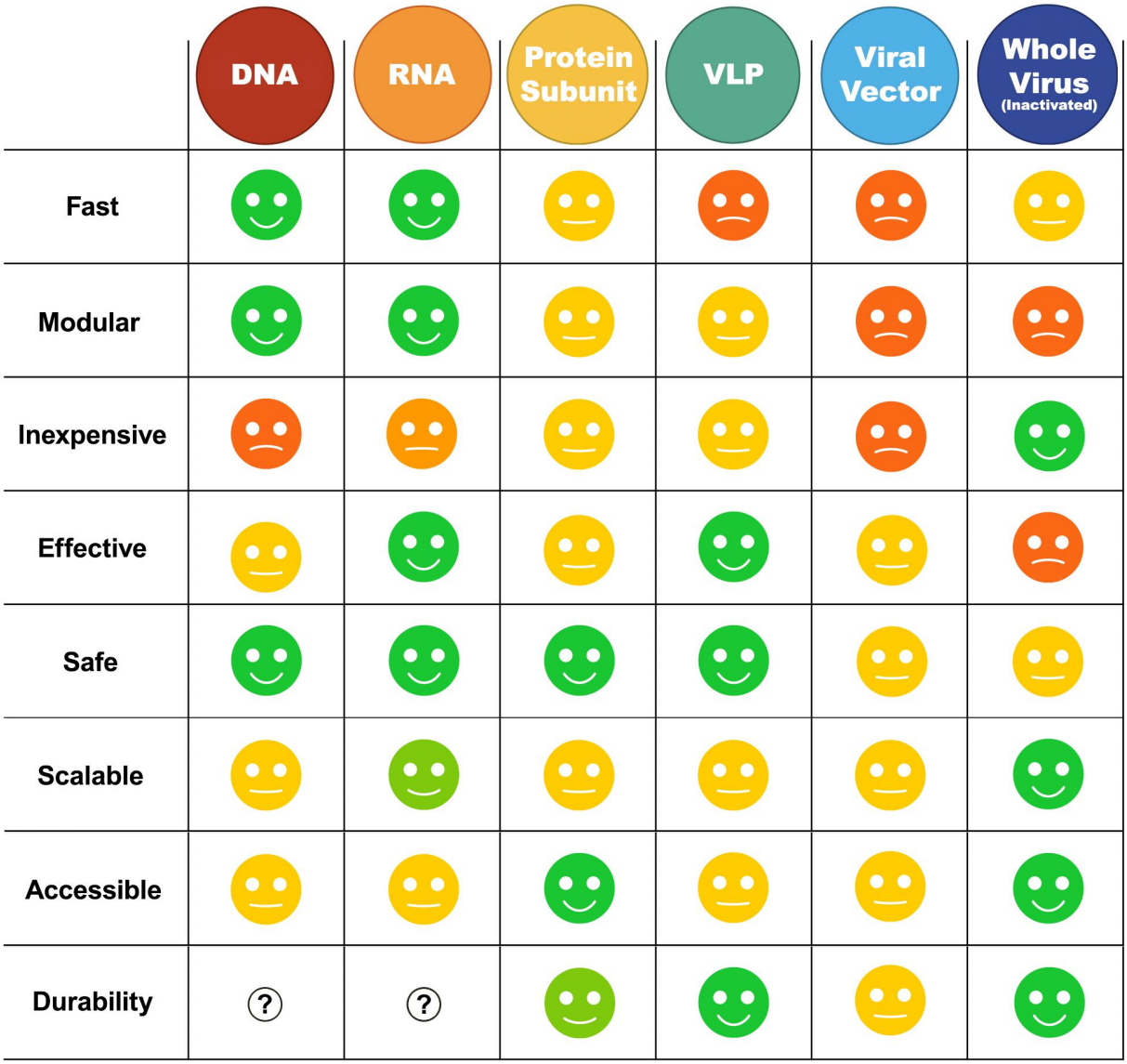
As of mid-February, 2022, 338 SARS-CoV-2 vaccines are under development in preclinical or clinical studies.[55] Five main types of vaccines have been deployed at scale, and continue to be developed:

- RNA
- Whole virus (attenuated or inactivated)
- Protein subunit (including virus-like particles)
- Viral vector
- DNA

Other approaches are also being investigated, including antigen-presenting cell–based vaccines, and even bacterial vectors.[56,57]

[54] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4586124/
[55] https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines
[56] https://bioscmed.com/index.php/bsm/article/view/473/
[57] https://clinicaltrials.gov/ct2/show/study/NCT05057923

Figure 1. A comparative analysis of documented attributes for six established vaccine platforms



Each of the main types of vaccine has different advantages and disadvantages:

RNA vaccines

RNA vaccines are a new and revolutionary class of vaccines, bringing many benefits over other vaccine types. One key advantage is that RNA vaccines are self-adjuvanting and do not seem to require additional adjuvants. This is very important, as adjuvants are often responsible for adverse effects of experimental vaccines, and are often a critical but unpredictable variable in the success or failure of an experimental vaccine.

Advantages:

- Fast and easy to design and redesign as needed
- Relatively easy to produce and scale
- Costs are declining rapidly
- Very effective immune response
- No additional adjuvant needed
- Very low probability of integration into the host genome
- Epitope/antigen selections are limited to specific portions of a virus, and can exclude known or suspected immunopathological sequences

Disadvantages:

- RNA is unstable and typically requires ultracold transport and storage, although there are early reports of stable formulations
- Currently expensive
- mRNA presently requires a moderately high dose (20 to 100 micrograms), although less might be required as technologies are optimized. Self-amplifying RNA (saRNA) has been shown in preclinical research to require very low doses, potentially in the sub-microgram range, for an effective immune response, although saRNA thus far triggers an innate immune response that limits efficacy

Whole virus

Certain viruses can be completely inactivated, or they can be attenuated or weakened by various means, and over many decades both have been used as vaccines to treat many diseases. Historically, inactivated virus vaccines generate weaker immune responses but are safer than attenuated virus vaccines.

Whole virus, attenuated

There are many approaches to attenuating viruses to create vaccines. Modern methods, such as codon deoptimization are not yet widely used, but result in vaccines that are much safer than older methods, such as serial cultivation or chemical attenuation. A virus attenuated by very precise means, such as codon deoptimization, can be used as an initial challenge agent, and also as a vaccine candidate. Below are other advantages and disadvantages of attenuated whole virus vaccines..

Advantages:

- Established technology
- Broad immune response
- Easy to manufacture

Disadvantages:

- May trigger the targeted disease in rare cases (mostly in the distant past)
- Variable effectiveness
- Careful storage needed
- Immunopathological sequences are present in every known virus

Whole virus, inactivated

Advantages:

- Established technology
- Broad immune response

Disadvantages:

- Relatively weak immune response
- May trigger the targeted disease in rare cases (mostly in the distant past)
- Variable effectiveness
- Possible/likely inclusion of immunopathological sequences

Protein/peptide subunit

Advantages:

- Established technology
- Shelf-life & shipping stability (depending on the design)
- generally effective (especially virus-like particles)
- Epitope/antigen selections are limited to specific portions of a virus, and can exclude known or suspected immunopathological sequences

Disadvantages:

- Relatively weak to moderate immune response, depending on adjuvant
- Protein subunit is often difficult to manufacture
- Peptide subunit vaccine is often quick and easy to manufacture, but is limited to simple epitopes
- Epitope/antigen selection is often laborious and time consuming

Viral vector

Viral vector vaccines are commonly used around the world. SARS-CoV-2 viral vector vaccines include the Johnson & Johnson / Janssen, the Oxford-AstraZeneca ChAdOx1, the Russian Sputnik, and CanSino. These widely used vaccines are all based on adenovirus vehicles.

Advantages:

- Relatively easy for existing facilities to scale, due to prior investment and existing infrastructure
- Moderately effective initial immune response

Disadvantages:

- Previous exposure to the vector could render it significantly less effective
- Relatively difficult to set up production
- Immunity can wane quickly (as occurred with widely used SARS-CoV-2 adenovirus vaccines)

DNA vaccines

Advantages:

- Fast and easy to design and redesign as needed
- Relatively easy to produce and scale
- Moderately effective immune response
- Epitope/antigen selections are limited to specific portions of a virus, and can exclude known or suspected immunopathological sequences
- Stable, even at room temperature

Disadvantages:

- High doses are required
- Moderately expensive
- Possible integration into the host genome

Ideally, all widely used or promising vaccine platforms would be tested in challenge trials, but there are practical limitations to such an approach. At least the top two or three promising platforms for a given pathogen should be included concurrently for trialing. The most promising platform to trial against SARS-CoV-2 would be an RNA vaccine. The two mRNA vaccines against SARS-CoV-2 in widespread use, made by BioNTech-Pfizer and Moderna, have proved to be about

95% effective against the virus in clinical trials[58],[59]. This level of effectiveness represents an extraordinary scientific achievement. The Johnson & Johnson/Janssen viral vector vaccine, JNJ-78436735, was shown to be only 66.3% effective at preventing disease,[60] and Sinovac's CoronaVac inactivated virus vaccine was merely 51% effective at preventing symptomatic disease,[61] barely above the commonly used threshold for regulatory approval.

Non-RNA vaccines may nonetheless prove to be advantageous under certain circumstances, and having a detailed understanding of how different types of vaccines compare to one another would be valuable in guiding local vaccine deployment decisions. For this reason, with sufficient resources, multiple trials ideally would be run in parallel: challenge trials with several different types of vaccines, along with conventional placebo-controlled randomized clinical trials. Each would examine not simply the obvious endpoint of the development of disease, but also detailed immunological changes, which would help tailor vaccine choice to particular individuals and populations. With sufficient variations on challenge trial designs – each examining correlates of protection in detail – and, perhaps most importantly, with parallel placebo-controlled randomized clinical trials, a kind of vaccine trial "Rosetta Stone" can be established, permitting translations between traditional RCTs and the faster, cheaper challenge trial designs. These translations would facilitate regulatory approval of challenge trials and in some contexts the eventual supersession of traditional clinical trials by more rapid, efficient, and safe challenge trials.

The immediate need, however, is to validate a vaccine platform that will permit rapid vaccine deployment, and, in order to be prepared for new SARS-CoV-2 variants of concern, rapid modification. In the absence of the hundreds of millions of dollars needed to run several parallel trials, the priority should thus be testing the most promising type of vaccine: RNA-based vaccines.

Pan-Coronavirus Vaccine (PanCoV214)

Vaccine design

As of early 2022, many public health officials have stated that SARS-CoV-2 is transitioning from pandemic to endemic. Concurrently, many publications are reporting three worrisome issues: the omicron variant has evolved substantial resistance to existing vaccines; recent omicron-specific booster vaccines have no greater efficacy against omicron than vaccines targeting the parental strain; and many who have received three total doses are experiencing symptomatic COVID-19.

[58] https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/Moderna.html
[59] https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/Pfizer-BioNTech.html
[60] https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines/janssen.html
[61] https://www.who.int/news/item/01-06-2021-who-validates-sinovac-[...]-issues-interim-policy-recommendations

Taken together, these facts suggest that ongoing evolution of further vaccine resistance is likely to produce even more serious disease, and use of the same vaccine strategies is unlikely to provide protection.

Certain general strategies are much more likely to yield positive results, and the vaccine that we suggest as a leading candidate for use in the step-up challenge trial is RaDVaC's broad-spectrum RNA vaccine, PanCoV214. Details of the RaDVaC RNA vaccine design strategy and rationale, including guidelines for the use of highly conserved epitopes in order to create broad-spectrum designs, can be found in the RaDVaC's vaccine white papers, including the recent mRNA vaccine white paper.[62]

Depending on prevailing conditions, of course, one might not choose to prioritize the testing of a broad-spectrum vaccine. The choice of vaccine to trial in a given location is determined by one of two prevailing conditions: preventive deployment, or emergency deployment in response to an outbreak. In preventive deployment, multiple criteria are used to select epitopes that are highly conserved and which play important roles in neutralization of the virus by the immune system. In an outbreak, the primary pathogen of concern should be the main target of the vaccine, with additional consideration given to broad-spectrum potential of highly conserved epitopes. Briefly, in either case, the vaccine will be based on the following primary criteria:

- Inclusion of epitopes engaged by the immune system, which elicit a response upon infection by the primary pathogen of concern. In natural infections, certain epitopes are shielded by various means, and these epitopes do not trigger an immune response (either B-cell or T-cell).
    - Inclusion of such shielded B-cell epitopes might require special engineering, such as unmasking[63] or immunofocusing.
    - It is unclear if similar approaches might be successful in forcing immune engagement of naturally undetectable T-cell epitopes.
- An ideal vaccine should include at least some epitopes that are highly conserved across the viral family (e.g. coronaviridae).
    - In general, B-cell epitopes (antibody binding) are less conserved than T-cell epitopes. This is especially true of immunodominant B-cell epitopes, such as the RBD of coronaviruses.
- Long-term durability. Most models of correlates of protection are focused on short-term outcomes. Long-term protection against severity of disease is extremely important yet

[62] https://radvac.org/white-papers (to be published by early April, 2022).
[63] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5021406/

often neglected. This short-term focus has resulted in many correlate of protection models focusing on antibodies, especially neutralizing antibodies. In coronaviruses, antibody and memory B-cell responses are typically undetectable within 4 years after initial infection, yet SARS-CoV-2 cross-reactive T-cell responses have been detected in convalescent patients 17 years after SARS-CoV-1 infection.

    - B-cell epitopes and neutralizing antibodies. It is frequently claimed that neutralizing antibodies are optimal correlates of protection, and that ideal vaccines target epitopes that elicit neutralizing antibodies. However, support for these claims has diminished over the course of the pandemic, and it is now clear that immunodominant neutralizing epitopes on the receptor binding domain have mutated sufficiently to greatly reduce or eliminate binding by neutralizing antibodies. Therefore, when durability of immunity is a goal, less immediately potent but conserved epitopes should be considered for protection against severe disease. This includes individually non- or weakly neutralizing epitopes that contribute synergistically to neutralization.

- All variant amino acids of a VOC do not need to be included in the vaccine design for the VOC to be neutralized, if other included epitopes combined provide substantial neutralization.
- A selected epitope should be a plausible candidate for appropriate immune engagement. A large fraction of viral proteins are recognized by antibodies, but many such engagements are incidental to infection and exposure of epitopes during cellular killing and clearance of a pathogen, and they do not contribute to neutralization of the pathogen. In SARS-CoV-2, the nucleocapsid protein has been suggested as a B-cell epitope, in part because it often produces the highest antibody titers of all CoV-2 proteins, although most models of the virus suggest that it resides in the interior of the viral particle. This is not unreasonable, as certain models suggest part of nucleocapsid might have at least intermittent exterior exposure. In contrast, it has been proposed that non-structural proteins such as nsp3 be used as B-cell epitopes, without any evidence that antibodies against these targets will contribute to pathogen neutralization.

A truly broad-spectrum vaccine should provide some protection against any emerging pathogen, even a zoonotic transmission. Therefore, the ongoing emergence of SARS-CoV-2 variants of concern should be within the scope of treatment/prevention capabilities of such a vaccine.

Details about the RaDVaC PanCoV214 can be found in the recent vaccine-specific white paper, but briefly, the following are key features of the vaccine:

- RaDVaC PanCoV214 is an mRNA and lipid nanoparticle vaccine, designed and produced with state-of-the-art components and technologies
- The vaccine consists of multiple, stabilized Spike glycoprotein S2 domains (the Spike stem or stalk), representing the range of observed variations across human and animal coronaviruses. This type of vaccine has emerged as a leading candidate for broad-spectrum vaccines, and has been tested in many different forms against influenza
- Additionally, the vaccine includes multiple highly conserved and/or immunodominant Class I and Class II T-cell epitopes, to enhance immune durability and to increase helper T-cell activation to promote a robust antibody response

Dosing and booster schedules

Commercial mRNA doses range from 30-100 µg. saRNA appears capable of eliciting a similar response with doses of just 1.25 µg,[64] and even as low as 0.05 µg, but with more variable efficacy than mRNA, possibly due to innate immune mechanisms.[65] Information shared by other researchers experimenting with PanCoV214 will help refine estimations of ideal dosing.

The original recommendations for spacing between first and second treatments of the FDA-approved mRNA vaccines were three weeks for Pfizer/BioNTech's COVID-19 vaccine and four weeks for Moderna's COVID-19 vaccine.[66] But later studies suggested that much longer intervals might elicit a stronger response in certain aspects of immunity. For example, an assessment of immune responses in the first 14 weeks of an extended-interval vaccination with Pfizer/BioNTech's vaccine showed that delaying the second dose boosts the peak antibody response 3.5-fold in older people.[67] Yet peak cellular-specific responses were the strongest in those vaccinated with the recommended 3-week vaccination interval. More research is clearly needed, and multiple vaccine trials with different booster schedules will begin to answer questions about ideal booster schedules.

Ongoing research elsewhere will of course also permit refinements of vaccine dose size and booster schedule. An adaptive trial would permit adding additional boosters.

[64] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5835025/
[65] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6953774/
[66] https://www.cdc.gov/coronavirus/2019-ncov/vaccines/second-shot.html
[67] https://pubmed.ncbi.nlm.nih.gov/35087066/

Vaccine administration

The prime dose of PanCoV214 will be administered parenterally. Booster dosing is possibly parenteral, mucosal, or both. Combining a parenteral prime dose and a mucosal booster in a so-called heterologous prime-pull (or prime-spike) regimen has been reported to create more extensive sterilizing immunity, including protection from infection in the upper respiratory tract.[68] RaDVaC is currently designing and testing administration of mucosal (nasal, sublingual, etc.) booster doses. Mucosal boosters are potentially self-administrable. In a trial context, this could be supported with live (possible via video) oversight. If an outpatient trial design is chosen, unsupervised self-administration of the boosters would be acceptable, after instruction and demonstrated success with the first treatment.

For a discussion of optimal protocols for vaccine administration, see the RaDVaC RNA vaccine white paper.[69]

Pathogen challenge administration: general considerations

Years of human challenge trial research, in particular influenza challenge trial research, can help guide decisions about important study parameters such as dosing and route of administration of the pathogen challenge. A meeting was convened in London in 2018 to review past influenza challenge studies and develop consensus recommendations for human influenza vaccine challenge studies. Table 3 below, from a report on the London meeting, describes the characteristics of different viral administration options for human influenza challenge trials.[70] This can help guide decisions about administration of other respiratory viruses, such as SARS-CoV-2.

[68] https://www.biorxiv.org/node/2368391.full
[69] https://radvac.org/white-papers (to be published by early April, 2022).
[70] https://www.sciencedirect.com/science/article/pii/S0264410X19308151?via%3Dihub

Table 3. Challenge virus delivery devices.

Route of Delivery	Device	Dose	Target Delivery Area	Residence Time	Droplet Size (µm)	Risk of Virus Escape	Other
Nasal			150 cm^2			Low	
	Droppers	High variability	Limited and variable; depends on positioning	Limited, variable			Simple to use
	Spray	Varies with angle/depth of spray	Increased		50–100		Can agglomerate into drops
	Gel			Increased			Requires additional formulation steps
	Aerosol	Varies depending on coverage/retention	Increased compared to spray	Variable	15–25		Dose delivered over time
	Dry powder	Less variable	Increased compared to liquid	Increased	25		Ongoing comparative delivery studies in ferrets

Route of Delivery	Device	Dose	Target Delivery Area	Residence Time	Droplet Size (µm)	Risk of Virus Escape	Other
Pulmonary			50–75 m2[71]			High	
	Liquid aerosol (including jet and pool-type) nebulizers		Large (alveolar)		<5		Requires time to administer; Can target upper or lower respiratory tract or both; may damage virus
	Vibrating mesh liquid aerosol nebulizer	More precise	Large (alveolar)		Specific droplet sizes possible		Licensed for asthma medications; used to deliver LAIV in ferrets and are being studied as part of the WHO Measles Aerosol Project.[72] Less damage to virus
	Dry powder inhaler	Less precise than nasal powders	Large (alveolar)		<5		Widely used for asthma medications

(LAIV: live attenuated influenza vaccine)

[71] https://www.sciencedirect.com/science/article/pii/S0264410X19308151?via%3Dihub#b0070
[72] https://www.sciencedirect.com/science/article/pii/S0264410X19308151?via%3Dihub#b0075

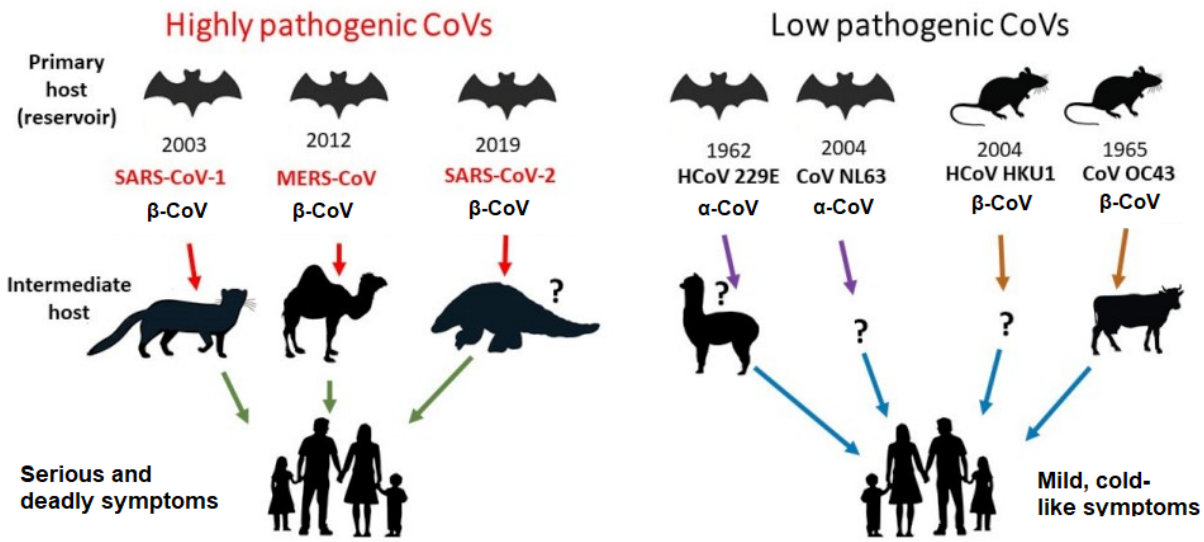
The consensus of the London meeting was that, for influenza challenge studies, a nasal atomizer is the best choice for delivery of challenge. The recommendation is based on a number of factors, including safety, large surface area delivery, and participant comfort. It should nonetheless be emphasized that, as with most elements of the step-up challenge trial design, optimal delivery route for the pathogen challenge is still partly an open question, and ongoing and future challenge studies, including step-up challenge trials, can contribute to more precise standards for all aspects of challenge trials.

Step 1 challenge virus

Choice of virus for step 1 challenge

There are seven known human coronaviruses, four of which typically cause only mild disease. See figure 2.

Figure 2.



The next coronavirus to cause a pandemic could come from any coronavirus genus. SpillOver, an open source, collaborative project that ranks viruses by risk of zoonotic transmission and potential for harm, currently lists well over a hundred coronaviruses – including three gammacoronaviruses – with the potential for spillover events to new host species, and at least

some chance of significant transmissibility and pathogenicity in humans.[73] Most of those listed as high-risk are in the betacoronavirus genus. Moreover, the three coronaviruses that have caused serious illness in humans have so far all been betacoronaviruses. For that reason, selecting a betacoronavirus as the step 1 challenge virus seems the most potentially productive choice, if the goal is to gather immune profile information on responses to likely potentially threatening coronaviruses, and to test a vaccine that might meet that threat. The use of a betacoronavirus in step 1 is also more likely to provide relevant immune profile information for the step 2 challenge with SARS-CoV-2. Of the two low pathogenic betacoronaviruses, HCoV-HKU1 and HCoV-OC43, HCoV-OC43 is better characterized, and thus should be easier to produce and use for the trial. There are several known HCoV-OC43 genotypes. Some may cause slightly more severe disease than others; nonetheless all appear generally to cause only mild disease in healthy people.[74] HCoV-NL63 seems to be somewhat more virulent and less common than HCoV-OC43. One major advantage to the use of HCoV-NL63 as a challenge agent is that it has essentially identical host cell spread, or tropism. HCoV-NL63 uses the human ACE2 receptor, and therefore infects the same range of host cells as both SARS-CoV and SARS-CoV-2.

Factors such as cost and complexity of production will factor into decisions about challenge viruses. By the time a particular trial is ready to initiate, well-characterized attenuated strains of (otherwise) highly pathogenic coronavirus strains might be available, including attenuated versions of SARS-CoV-2. Some researchers may want multiple arms with several steps, or challenges running in parallel.

Dosing and administration

In previous non-human primate studies of coronavirus infection and vaccination, viral challenge dose is 7.6×10^5 plaque-forming units (PFU) of SARS-CoV-2 per animal (approximately equivalent to 10^6 50% tissue-culture infectious doses [$TCID_{50}$])[75]. As many as 10 animals per study arm have been used, stratified by sex, age, and weight[76], but prior studies have used as few as two animals per group[77].

A similar dose of HCoV-NL63 would presumably be a reasonably safe dose for the determination of pathogenicity, but disease course and viral kinetics may be very different in previously unexposed humans, hence we suggest a lower dose.

[73] https://www.pnas.org/content/118/15/e2002324118
[74] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3194943/
[75] https://www.nejm.org/doi/full/10.1056/NEJMoa2024671
[76] https://www.nejm.org/doi/suppl/10.1056/NEJMoa2024671/suppl_file/nejmoa2024671_appendix_2.xlsx
[77] https://www.nature.com/articles/s41422-020-0364-z

For the low-dose step 1 challenge, stock solution of 1.9×10^4 PFU per milliliter HCoV-NL63 administered in a volume of 1.5 ml by the intratracheal route and in a volume of 0.5 ml by the intranasal route (0.25 ml per nostril), four weeks after vaccination. For the high-dose challenge, the dose will be doubled.

Step 2 challenge virus

Choice of virus for step 2 challenge

Many SARS-CoV-2 variants of concern (VOC) have arisen globally. Certain mutations, such as N501Y, are seen in multiple VOC including B.1.1.7 [Alpha], B.1.351 [Beta], and the P.1 variant [Gamma] of the B.1.1.28 lineage, and the more recent B.1.1.529, BA.2, and BA.2 [Omicron family]. Nevertheless, more VOC will arise and the situation will remain highly dynamic for the foreseeable future, with many changes occurring in the list of predominant strains. For a trial to produce results for a relevant vaccine, predictions must be made at the outset of the trial about which VOC will likely be predominant. A truly broad-spectrum vaccine should provide some protection against any emerging related pathogen, including zoonotic spillover events, but which strain to choose for a challenge will depend on local conditions and predictions.

Dosing and administration

Previous non-human primate studies of coronavirus infection and vaccination may not be applicable to dosing estimates for humans of SARS-CoV-2. Indeed, the World Health Organization recommended, early on in the pandemic, an ideal starting challenge dose of 10^2 $TCID_{50}$.[78]

Results of the hVIVO human SARS-CoV-2 challenge trial have now been made available as a (not yet peer-reviewed) preprint.[79] Remarkably, the hVIVO trial found that a dose of just 10 $TCID_{50}$ was sufficient to meet a target infection rate of 50-70%.

For the low-dose step 2 challenge, we suggest following the hVIVO dosing. For a high-dose challenge, the dose should be doubled, which still be well below the conservative W.H.O. recommendation.

[78] https://www.ncbi.nlm.nih.gov/labs/pmc/articles/PMC7499532/
[79] https://www.researchsquare.com/article/rs-1121993/v1

Immunobridging: Immune profiling and other data analyses

Introduction

We propose a systems vaccinology approach, combined with bioinformatics, to generate highly detailed correlates of immunity, as well as correlates of immunological durability.

Establishing correlates of immunity will have a number of benefits. One, specific to the step-up trial design, is that we will have a very precise idea of which high-risk candidates for step 2 of the trial are least likely to suffer harm from the viral challenge, and can be permitted to participate in step 2. Detailed correlates of immunity will also increase the power of the study.

Beyond the specific advantages to the step-up trial, there is enormous potential scientific value in a fine-grained determination of immune correlates of protection. There are a number of important questions about immune response to vaccines, and immunity in general, that trials following this design can help answer. Knowledge might be gained in many important areas: Which profiles – genotypes, molecular signatures, immune cell type changes, clinical markers, etc. – correlate with high reactogenicity, including serious adverse events? Which profiles correlate with strong immunity? How do these profiles vary by age, gender, and other factors? And of course: Which profiles correlate with a strong response to a particular vaccine, or even vaccines in general?

There are many populations with a high proportion of people suffering from lifestyle diseases and ill-health (overweight, metabolic dysfunction, etc.). Principal investigators in all locations will of course need to examine volunteers and exclude those at high risk of severe COVID-19 disease. But, especially once better rescue therapies are in place, some people with non-severe lifestyle disease could enroll relatively safely in a step-up challenge trial. This could lead to the discovery of factors that could be protective even in those with, for example, diabetes or other types of metabolic dysfunction. Including such volunteers in the study might also shed light on precisely why it is that those with lifestyle-related illness are more prone to severe COVID-19 disease.

Deep immune profiling

Deep immune profiling refers to the integrated assaying of a wide array of immunological factors (e.g. specific antibodies, cytokines, T- and B-cell populations, genomics, and more), for the purpose of comprehensively characterizing the activity/response of an individual's immune system. This systems-level approach to immunology, when applied in analyses of groups of individuals, has the power to reveal biomarkers and molecular networks of clinical importance which, if assayed only individually, remain mostly opaque to researchers.

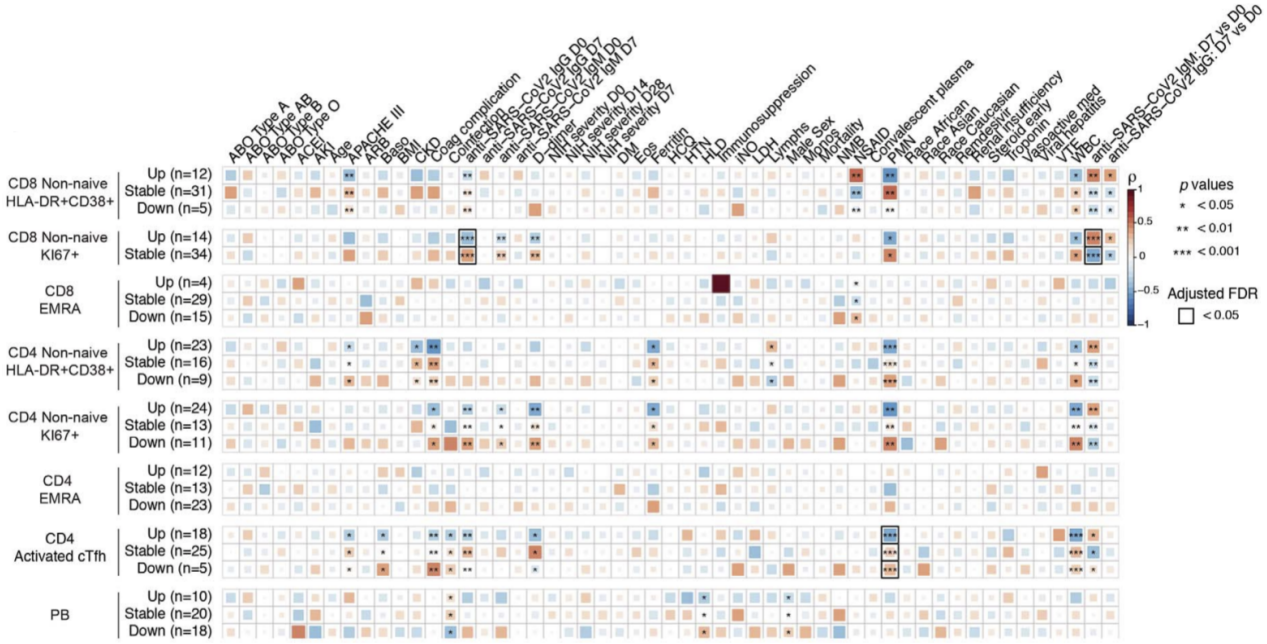
There is a vast array of determinants and correlates of immunological function for every individual, including hundreds of classes and subclasses of immune cells, thousands of interrelated immune signaling molecules, three major Human Leukocyte Antigen (HLA) types, a dozen known HLA isoforms, encoding hundreds of HLA proteins involved in antigen presentation to T-cells. Table 4, below, represents correlations between just eight cell types with other measurements thought possibly to be related to COVID-19 disease course.

Immunologists and related organizations have expressed the urgent need to determine the most important biomarkers for understanding and predicting clinical progression of COVID-19, and the mechanics of SARS-CoV-2 infection and immunology more fundamentally. Understanding how these biomarkers relate to immune protection is a necessary prerequisite for vastly more efficient, rapid, and statistically powerful vaccine trials, including immunobridging studies, leveraging predictive biomarkers rather than relying on incidental, clinically identifiable infection in the study arms. As of March 2022, there is not yet an accepted immunological correlate of protection for COVID-19[80,81], and no immunobridging studies have yet been established to eliminate the reliance on the present standard of placebo-controlled randomized clinical trials.

Finding useful correlations in high-dimensional data presents enormous challenges.[82] Feature selection can make the problem more tractable[83] but, ultimately, machine learning will likely be needed to make optimal use of extremely high-dimensional data. Multiple correlate of protection models have been developed for SARS-CoV-2 vaccination (e.g. [84],[85]), but none has been developed for a broad-spectrum vaccine or for SARS-CoV-2 challenge trials. Nevertheless, existing modeling approaches should be adapted readily for these specific needs. Since late 2019 there has been an unprecedented amount of research into COVID-19-related pathology and immune response characterization, which will continue to yield insights and reveal immune mechanisms and markers that are yet not known or understood. Long-term biobanking of participant samples for later analysis using assays developed in the future is therefore an important step toward maximizing trial findings, and for comparability with results from subsequent trials.

[80] https://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/summary-2021-05-12-508.pdf
[81] https://cdn.who.int/media/docs/default-source/blue-print/final-agenda-immunobridging.pdf
[82] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2865881/
[83] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4581890/
[84] https://www.nature.com/articles/s41467-022-28898-1
[85] https://pubmed.ncbi.nlm.nih.gov/34812653/

Table 4. Spearman correlations of clinical parameters with longitudinal fold changes in immune populations.[86]



Identifying Correlates of Protection, and Correlates of Protection Durability: biological samples, clinical measures

Resource limitations might make deep immune profiling impractical for certain research groups. Below are suggestions based on the existing body of research being considered by the World Health Organization's R&D Blueprint working group, regarding the creation of immunobridging study standards[87,88]. We propose that these metrics will enable, when multiplexed, the establishment of useful immunity-related correlations.

[86] https://www.science.org/doi/pdf/10.1126/science.abc8511
[87] https://cdn.who.int/media/docs/default-source/blue-print/final-agenda-immunobridging.pdf?sfvrsn=8e074908_17
[88] https://cdn.who.int/media/docs/default-source/blue-print/who-cop_3sept2021_v3.pdf?sfvrsn=a20d5d39_7

Baseline only

- Whole genome sequencing, ideally using long-read technologies (for example, Oxford nanopore), which are rapidly maturing to the point of enabling accurate HLA allele characterization[89]
- Medical history
- Sample collection for biobanking (whole blood, PBMC isolation)

Baseline and ongoing

- Antigen dependent assays:
    - Interferon-g release assay
        - Easy, fast, commercially available
        - No information on phenotype
    - Interferon-g ELISPOT
        - Scalable to larger numbers
        - Little information on phenotype
    - Activation-induced marker
        - Sensitive, phenotypes of specific cells
        - Labor-intensive, no functionality
    - Intracellular cytokine staining (ICS)
        - Sensitive, phenotype and functionality info
        - Labor-intensive
    - Neutralizing antibody assays – both HCoV-NL63 (or similar low-virulence coronavirus strain) and SARS-CoV-2[90]
        - To assess mucosal immune response, secretory IgA (sIgA) should be assayed[91]
    - Binding, non-neutralizing antibody assays – both HCoV-NL63 (or similar low-virulence coronavirus strain) and SARS-CoV-2[92]
        - To assess mucosal response, sIgA should be assayed.

[89] https://pubmed.ncbi.nlm.nih.gov/33612390/
[90] https://www.sciencedirect.com/science/article/pii/S0092867420316858
[91] https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-3083.1977.tb00366.x
[92] https://cdn.who.int/media/docs/default-source/blue-print/composition_galit-alter[...]23feb2022.pdf

    - Virus-specific T-cell characterization (ELISpot[93], transcriptomic profiling of PBMC[94,95], flow cytometry, etc.)
        - Labor intensive
        - Determining tissue-specific T-cell population can be challenging (involves tissue collection)
    - Virus-specific B-cell characterization (ELISpot, flow cytometry)
- Antigen-independent assays:
    - Tetramer stainings[96]
        - No antigen variability
        - HLA / epitope dependent
    - T-Cell Receptor (TCR) sequencing[97]
        - Standardizable, scalable to large number
        - No information on specificity / function
    - Complete physical examination
    - Complete blood count (CBC) and blood chemistry panels (metabolic, lipid, cardiac, coagulation, thyroid, C-reactive protein)
    - Quantitative PCR to determine viral titer and shedding (nasal, throat, rectal samples)
    - Blood oxygen saturation
    - Tests for anosmia/hyposmia; and for ageusia/hypogeusia

Summary

Challenge trials have the power to accelerate vaccine approval, compared to the current accepted standard of placebo-controlled randomized clinical trials. Here we propose a novel challenge trial model (step-up challenge trial) to assess vaccine efficacy across multiple related pathogens. Trialing and regulatory approval for a broad-spectrum vaccine allows for the establishment of safety data and correlates of protection models for the vaccine, which should enable rapid secondary efficacy testing and deployment upon future outbreak of a related zoonotic pathogen.

Ultimately, the rationale for the step-up design is based on the following three key points

[93] https://en.wikipedia.org/wiki/ELISpot
[94] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3946932/
[95] https://www.ncbi.nlm.nih.gov/pubmed/?term=30205979
[96] https://bcmd8.bcm.edu/research/atc-core-labs/mhc-tetramer-production/suggested-staining-protocol
[97] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6058020/

- Information is needed on high-virulence pathogens, but also on related and common lower-virulence pathogens. Integrated challenge trials for multiple members of pathogen families will provide key details for understanding similarities and differences in immune responses to each pathogen, and it makes sense to trial them in order of ascending virulence, according to a standard protocol.
- The emerging consensus view that broad-spectrum vaccines for various pathogen groups/families are both possible and highly desirable.
- Correlates of protection derived from low virulence studies potentially will enable higher-virulence challenge of participants in high-risk groups (older or with pre-existing conditions) predicted to be protected by broad-spectrum vaccination. Understanding infection and protection in those at high risk is critically important, and current challenge trial designs avoid controversy by allowing challenge of only low-risk participants, and thus do not meet this need.

If we are to prevent worldwide spread and establishment of future pandemic pathogens, it is essential that vaccine deployment be far more rapid than the one-year record-setting performance in 2020. The most effective–and possibly only–means for doing so, is to have a pre-tested and very likely effective broad-spectrum vaccine ready for rapid deployment. The challenge trial design presented here is uniquely tailored for the safe and rapid testing of promising vaccine candidates for this purpose.

Trial Design Schematic

Figure 3.