
Article:
Cable, Jennifer, Rappuoli, Rino, Klemm, Elizabeth J. et al. (31 more authors) (2022) Innovative vaccine approaches—a Keystone Symposia report. Annals of the New York Academy of Sciences. pp. 59-86. ISSN 0077-8923

https://doi.org/10.1111/nyas.14739

Ann. N.Y. Acad. Sci. ISSN 0077-8923

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Special Issue: *Keystone Symposia Reports*

Concise Original Report

Innovative vaccine approaches—a Keystone Symposia report

Jennifer Cable,[1] Rino Rappuoli,[2] Elizabeth J. Klemm,[3] Gagandeep Kang,[4] Ankur Mutreja,[5] Gavin J. Wright,[6,7] Mariagrazia Pizza,[2] Sowmya Ajay Castro,[8] Joseph P. Hoffmann,[9] Galit Alter,[10,11] Andrea Carfi,[12] Andrew J. Pollard,[13] Florian Krammer,[14] Ravindra K. Gupta,[5,15] Caroline E. Wagner,[16] Viviane Machado,[17] Kayvon Modjarrad,[18] Lawrence Corey,[19,20,21] Peter B. Gilbert,[21] Gordon Dougan,[5] Nicole Lurie,[22,23] Pamela J. Bjorkman,[24] Christopher Chiu,[25] Elisa Nemes,[26] Stephen B. Gordon,[27] Andrew C. Steer,[28,29,30] Thomas Rudel,[31] Catherine A. Blish,[32,33] John Tyler Sandberg,[34] Kiva Brennan,[35] Keith P. Klugman,[36] Lynda M. Stuart,[37,38] Shabir A. Madhi,[39] and Christopher L. Karp[38]

[1]PhD Science Writer, New York, New York. [2]GSK, Siena, Italy. [3]Wellcome Trust, Bloomsbury, London, UK. [4]Division of Gastrointestinal Sciences, Christian Medical College, Vellore, India. [5]Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID) and Department of Medicine, University of Cambridge, Cambridge, UK. [6]Cell Surface Signalling Laboratory, Wellcome Sanger Institute, Hinxton, UK. [7]Department of Biology, Hull York Medical School, and York Biomedical Research Institute, University of York, York, UK. [8]Division of Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee, UK. [9]Departments of Pediatrics and Medicine, Center for Translational Research in Infection and Inflammation, Tulane University School of Medicine, New Orleans, Louisiana. [10]Ragon Institute of MGH, MIT and Harvard, Harvard Medical School, Cambridge, Massachusetts. [11]Division of Infectious Diseases, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts. [12]Moderna Inc., Cambridge, Massachusetts. [13]Oxford Vaccine Group, Department of Paediatrics, University of Oxford and the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre, Oxford, UK. [14]The Tisch Cancer Institute and Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, New York. [15]Africa Health Research Institute, Durban, South Africa. [16]Department of Bioengineering, McGill University, Montreal, Quebec, Canada. [17]Measles and Respiratory Viruses Laboratory, WHO/NIC, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil. [18]Emerging Infectious Diseases Branch, Walter Reed Army Institute of Research, Silver Spring, Maryland. [19]Department of Laboratory Medicine and Pathology, University of Washington School of Medicine, Seattle, Washington. [20]Department of Medicine, University of Washington School of Medicine, Seattle, Washington. [21]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington. [22]Coalition for Epidemic Preparedness Innovations, Oslo, Norway. [23]Harvard Medical School, Boston, Massachusetts. [24]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California. [25]Department of Infectious Disease, Imperial College London, London, UK. [26]Division of Immunology, Department of Pathology, South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa. [27]Malawi Liverpool Wellcome Research Programme, Blantyre, Malawi. [28]Infection and Immunity, Murdoch Children's Research Institute, Parkville, Victoria, Australia. [29]Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia. [30]Department of General Medicine, The Royal Children's Hospital, Melbourne, Victoria, Australia. [31]Microbiology Biocenter, University of Würzburg, Würzburg, Germany. [32]Division of Infectious Diseases and Geographic Medicine, Department of Medicine, Stanford Immunology Program, Stanford University School of Medicine, Stanford, California. [33]Chan Zuckerberg Biohub, San Francisco, California. [34]Department of Medicine Huddinge, Center for Infectious Medicine, Karolinska Institutet, Stockholm, Sweden. [35]National Children's Research Centre, Crumlin and School of Medicine, Trinity College Dublin, Dublin, Ireland. [36]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, Georgia. [37]Immunology Program, Benaroya Research Institute at Virginia Mason, Seattle, Washington. [38]Bill & Melinda Gates Foundation, Seattle, Washington. [39]South African Medical Research Council Vaccines and Infectious Diseases Analytics Research Unit, University of the Witwatersrand, Johannesburg, South Africa

Address for correspondence: annals@nyas.org

doi: 10.1111/nyas.14739

The rapid development of COVID-19 vaccines was the result of decades of research to establish flexible vaccine platforms and understand pathogens with pandemic potential, as well as several novel changes to the vaccine discovery and development processes that partnered industry and governments. And while vaccines offer the potential to drastically improve global health, low-and-middle-income countries around the world often experience reduced access to vaccines and reduced vaccine efficacy. Addressing these issues will require novel vaccine approaches and platforms, deeper insight how vaccines mediate protection, and innovative trial designs and models. On June 28–30, 2021, experts in vaccine research, development, manufacturing, and deployment met virtually for the Keystone eSymposium "Innovative Vaccine Approaches" to discuss advances in vaccine research and development.

Keywords: correlates of protection; COVID-19; human challenge studies; influenza; pneumococcal disease; streptococcus; vaccines; vaccine equity

Introduction

One of the key successes that emerged from the COVID-19 pandemic response was the speed at which highly effective vaccines were developed, tested, and approved for use. Unprecedented, these efforts leveraged decades of research into novel vaccine platforms and technologies and have potentially changed the field of vaccinology forever. At the same time, the vaccine distribution efforts exposed a lack of accountability globally to ensure equitable vaccine distribution among low-to-middle income countries (LMICs). Efforts to ensure vaccination coverage to the ongoing, ever-changing COVID-19 pandemic, and to address pathogens currently unamenable to vaccines, will require applying and adapting what has been learned throughout the pandemic in the context of novel vaccine platforms, deeper insights into the immune response, new models to investigate vaccine efficacy, and concerted global efforts to facilitate vaccine development, manufacturing, and distribution in LMICs.

On June 28 to 30, 2021, experts in vaccine research, development, manufacturing, and deployment met virtually for the Keystone eSymposium "Innovative Vaccine Approaches" to discuss advances in vaccine research and development. A large part of the meeting focused on COVID-19 vaccines, including how changes in vaccine research, development, and regulatory review led to the swift approval of several COVID-19 vaccines. Speakers also discussed how COVID-19 vaccine efficacy may be impacted by emerging SARS-CoV-2 variants and how emerging data in structural vaccinology and computational modeling can inform vaccination strategies, such as boosters and developing pan-coronavirus vaccines.

The symposium also focused on vaccine research for pathogens prevalent in LMICs. While vaccines are available for several important pathogens, such as rotavirus and *Mycobacterium tuberculosis*, they have not been effective enough to eliminate the global burden of these diseases. In addition, many unanswered questions remain about why some vaccines are more effective at eliciting immune responses and protecting against disease in high-income countries compared to LMICs. At the same time, there are many pathogens that have proven intractable to vaccines and impart significant socioeconomic burden on LMICs. Recent developments in novel vaccine platforms, reverse vaccinology, and systems immunology may offer novel approaches for effective vaccines, while changes to clinical trial paradigms, such as human challenge studies, may accelerate the vaccine development timeline.

Keynote address: 10 months to a COVID-19 vaccine—how did we get here?

Rino Rappuoli from GSK presented an overview of how vaccine development has changed over time and how changes to the clinical development process enabled the fastest vaccine development ever in 2020 to combat COVID-19. In many ways, the rapid development of COVID-19 vaccines marks a turning point in vaccine development. According to Rappuoli, "We are at an inflection point in the history of vaccines. One of those points of no return…, vaccines and vaccination will never be the same."

Rappuoli recalled how during the 2009 H1N1 influenza pandemic vaccines were developed too late to have a major impact on the course of the pandemic. In 2012, Rappuoli proposed[1] ways to shrink timelines for vaccine discovery and development by 2020. At the time, advances in technology, including genomics, reverse vaccinology, and new adjuvants, had considerably shortened the discovery timeline for vaccines. These technologies allowed researchers to test multiple approaches in parallel, markedly shortening the time to identify the best candidate vaccines and formulations. At the same time, the clinical development timeline had increased dramatically compared to previous decades; for example, clinical trial sample sizes had grown so that a vaccine candidate entering phase 1 trials could require 10 years of clinical testing. Rappuoli proposed that parallelizing the development process as well could shorten the clinical development timeline.[2] This was realized in 2020 during the development of COVID-19 vaccines.

Going into 2020, several new technologies were available to shorten the vaccine development timeline, including the incorporation of new biomarkers into phase 3 studies; however, predictions remained that full vaccine development would still take 8–9 years. Rappuoli credited four main advances with enabling faster development of the COVID-19 vaccines: *structural vaccinology*, that is, using structural data to improve, stabilize, or engineer antigens; *synthetic biology*, that is, the ability to make synthetic genes for RNA-based vaccines; *adjuvants*; and *internet-based* or *digital vaccines*. By internet-based vaccines, Rappuoli meant the dissemination of viral genome sequences around the world to enable researchers to develop vaccines without ever having the physical virus in their possession. This was first accomplished in 2013 when the Chinese CDC uploaded the sequence of an influenza H7N9 avian strain believed to have pandemic potential. Using the sequence, researchers synthesized viral genes and created a synthetic virus and RNA vaccine that was ready for preclinical studies within 1 week.[3,4] Similarly, publication of the SARS-CoV-2 genome[5] in early 2020 enabled researchers to quickly mine the genome for likely antigens and develop synthetic genes. Ultimately, vaccines were developed using three main modalities. Vaccines using viral mRNA entered clinical trials approximately 2 months after the genome was available, while adenovirus-based vector and protein-based vaccines entered clinical trials approximately 3 and 6 months, respectively, after the SARS-CoV-2 genome was available.

Despite these technological advances, the rapid development of the COVID-19 vaccines would not have been possible without significant financial support from the public sector. As in clinical development of other clinical modalities, vaccine development typically consists of sequential preclinical, phase 1, phase 2, and phase 3 studies, with each phase being more costly than the last. Companies are understandably hesitant to take on the financial risk of investing in later, more expensive, stages of development until a candidate has succeeded in earlier studies. However, for the COVID-19 vaccines, investments by governments worldwide shifted the financial risk from industry to the public sector, enabling companies to parallelize the development process and conduct later-stage trials before earlier studies were complete. This ultimately shrank the vaccine development process from 20 years to 10 months. Rappuoli stressed that this remarkable reduction in development time had no effect on steps necessary to assess vaccine safety and efficacy. Such technological improvements and public sector investments made it possible for vaccines to be available in time to alter the course of the SARS-CoV-2 pandemic: by producing sufficient neutralizing antibodies (nAbs) to provide protection from symptomatic SARS-CoV-2 infection.[6]

One of the key questions at the time of the Keystone symposium was whether vaccines would similarly protect against emerging variants. The evidence so far suggests that vaccine-induced immunity is stronger than natural immunity and will likely be able to defend against new variants (with additional booster doses). In addition, hybrid

immunity, which occurs when natural immunity is combined with vaccine-generated immunity, may provide even greater protection than either form alone, suggesting that there is much to be hopeful about as vaccination rates continue to rise.[7] This hope continues to be so even in the face of new strains of SARS-CoV-2, such as the Omicron strain first reported in South Africa in November of 2021.

COVID-19 vaccines: efficacy against viral variants

When the COVID-19 pandemic began, vaccine research on coronaviruses was already well established. Two prior outbreaks, SARS in 2002 and MERS in 2012, had raised the alarm that coronaviruses had the potential for pandemic spread. While these outbreaks resolved without the need for vaccines, they helped to drive research that identified the spike protein as a key target for protective immune responses. In fact, a phase 1 trial of a viral vector vaccine that expresses MERS spike protein had been published in mid-2020, months before SARS-CoV-2 emerged.[8] Therefore, when the SARS-CoV-2 genome was released around the world in early 2020, researchers were equipped with both the knowledge and tools to quickly develop a vaccine. Currently, several COVID-19 vaccines are available that use the spike protein as the target of immune responses. Several speakers discussed the development and efficacy of COVID-19 vaccines, as well as protection against SARS-CoV-2 variants.

The Moderna mRNA COVID-19 vaccine, mRNA-1273

Andrea Carfi from Moderna described the development of Moderna's mRNA COVID-19 vaccine and the company's approach to preparing for emerging variants of concern. Moderna's vaccine consists of a codon-optimized mRNA that codes for the SARS-CoV-2 spike protein, surrounded by a lipid nanoparticle that protects the mRNA from degradation, delivers it to cells, and facilitates mRNA escape from the endosome after the nanoparticle has been taken up by cells. Lipid nanoparticles are taken up by antigen presenting cells (APCs) at the site of administration and in draining lymph nodes. APCs then express and present a viral spike protein epitope to immune cells, thus initiating an adaptive immune response. As a vaccine platform, mRNA is well suited for pandemic responses, as vaccines can easily be modified or updated by changing the mRNA sequence to that of a new antigen; the manufacturing process and reagents remain the same. While the COVID-19 mRNA vaccines were the first used widely in humans, Carfi stressed that Moderna has invested in this technology for over 10 years and, prior to the SARS-CoV-2 pandemic, had conducted several clinical studies targeting respiratory viruses that demonstrated both the immunogenicity and tolerability of Moderna mRNA vaccines.

When the SARS-CoV-2 genome was released in early 2020, Moderna designed and optimized an mRNA vaccine encoding the SARS-CoV-2 pike protein; an NIH-sponsored phase 1 study started approximately 2 months later. Carfi stressed that funding from sources such as the Biomedical Advanced Research and Development Authority (BARDA), and close interactions with regulatory agencies, enabled Moderna to accelerate the development timeline. Interim phase 3 study results were available in November 2020. The vaccine received FDA emergency use authorization in December 2020 and European Medicines Agency (EMA) conditional marketing authorization in January 2021.

In preclinical studies, the vaccine induced robust antibody responses that inhibited the interaction between the viral spike protein and the ACE2 receptor, and protected against viral replication. In the phase 1 clinical trial conducted in 120 individuals, the vaccine elicited comparable nAb titers across age groups, as well as a T_H1 $CD4^+$ T cell response.[9] The phase 3 study was a placebo-controlled trial conducted in over 30,000 adults in the United States. The vaccine demonstrated 94% efficacy in the primary endpoint of preventing symptomatic COVID-19, as well as 100% efficacy in preventing severe disease.[10] Data in adolescents showed similarly high efficacy. A separate study (KidCOVE Study) is underway to test the safety, tolerability, and efficacy of mRNA-1273 in individuals 6 months to 11 years of age (https://clinicaltrials.gov/ct2/show/NCT04796896).

One of the primary concerns for vaccines at this point is how well they protect against emerging variants. Sera from subjects in the mRNA-1273 phase 1 trial showed that several variants demonstrate a decrease in neutralizing response. Moderna has updated its vaccine to target variants of concern. A phase 2 trial is underway

to investigate several boosting strategies in individuals fully vaccinated with mRNA-1273: a boosting dose against the B.1.351 (beta) variant (mRNA-1273.351), a boosting dose with a multivalent vaccine against both the original SARS-CoV-2 and the beta variant (mRNA-1273 + mRNA1273.351), and a boosting dose with mRNA-1273. In mice, boosting with mRNA-1273.351 produced a comparable neutralizing response against both the original strain and the beta variant.[11] Studies ongoing in mice, hamsters, and nonhuman primates (NHPs) are underway to evaluate boosting with monovalent mRNA-1273 or mRNA1273.351 and multivalent mRNA.1273.211. Preliminary data in humans show that a boosting dose of either mRNA-1273 or mRNA-1273.351 increased the nAb response against all variants tested.[12]

The Oxford viral vector vaccine ChAdOx-nCov9

Andrew J. Pollard from the University of Oxford reviewed the development of the Oxford viral vector vaccine, ChAdOx-nCoV9. Phase 1 and 2 clinical studies in early 2020 demonstrated the benefit of a two-dose regimen in eliciting nAbs, as well as a strong T cell response. Data in older adults (>55 years) showed that two doses elicited similar antibody titers as younger participants.[13,14] Phase 3 trials across the UK, Brazil, and South Africa showed that the vaccine had 100% efficacy in preventing hospitalization, severe disease, and death as well as high efficacy against mild infection.[15] The vaccine was authorized in the UK in December 2020. Since then, real-world data demonstrate that the vaccine has high effectiveness against hospitalization in all age groups.[16,17] These data have informed the vaccine rollout strategy in the UK, which prioritized offering individuals first doses.[18] Additional studies are ongoing in collaboration with AstraZeneca in the United States, Russia, Japan, and India. In total, almost 60,000 subjects were included across the phase 3 program. Pollard stressed that, although the timeline from first dose to authorization was significantly shorter than in the past, the clinical development and regulatory processes remained robust. Similar to other vaccines, vaccine efficacy against SARS-CoV-2 infection and mild disease wanes as new variants emerge; however, real-world data with the delta variant have so far suggested that the vaccine provides high protection against hospitalization and severe disease.[19,20]

One of the key goals of the Oxford Vaccine Group was to ensure broad global equity in vaccine access. Pollard noted that their partnership with AstraZeneca provides a global manufacturing network and established supply capacity to enable broad, equitable access worldwide. While there is clearly considerable inequity between high- and low-income countries in terms of vaccine access, the Oxford-AstraZeneca vaccine is currently the most widely distributed and has been administered in over 180 countries, including many countries in Africa that do not have access to other vaccines.[21]

INO-4800, a DNA vaccine for COVID-19

Viviane Machado from Inovio Pharmaceuticals presented immunogenicity data on INO-4800, a DNA vaccine for SARS-CoV-2 variants. DNA vaccines consist of the DNA sequence of an antigen of interest incorporated into an expression vector and administered into the muscle or skin via electroporation. Transfected cells express the antigen, generating an immune response. INO-4800 contains the DNA sequence of the SARS-CoV-2 spike protein. Since DNA vaccines mimic natural infection in harnessing the host machinery for antigen production, it is hoped that they will induce a similar immune response as natural infection as well. In a phase 1 trial, INO-4800 was shown to be safe and tolerable and to generate neutralizing and cellular responses.[22] Preliminary phase 2 study[23] has been reported, and a phase 3 is being planned.[24]

Both animal data and samples from subjects immunized in the phase 1 trial indicated that INO-4800 induces both cellular and humoral immune responses against the alpha, beta, and gamma SARS-CoV-2 variants, generating comparable IgG antibody titers. It also generated neutralizing responses, though there was a modest reduction in neutralization titers for alpha and beta variants. T cell responses were maintained as well in vaccinated individuals.[24,25] In a challenge study, INO-4800 protected hamsters from body weight loss after challenge with the beta variant (see https://doi.org/10.1101/2021.05.11.443592).

A SARS-CoV-2 ferritin nanoparticle vaccine

Kayvon Modjarrad from Walter Reed Army Institute of Research presented the efficacy of

a SARS-CoV-2 ferritin nanoparticle vaccine in NHPs. Ferritin is a ubiquitous protein that self-assembles into a polymer. To create a vaccine, antigens are linked to ferritin monomers, and the monomers self-assemble into nanoparticles. Ferritin-based influenza vaccines that incorporate the hemagglutinin (HA) stem have demonstrated broad neutralization in clinical trials.[26,27] Modjarrad's group is investigating the use of ferritin nanoparticles to address multiple viral pathogens to elicit protection against a range of viruses within a given family.

Modjarrad described preclinical results for a SARS-CoV-2 spike ferritin nanoparticle (SpFN) administered with ALFQ, a liposomal adjuvant containing MPLA and QS-21. The group has also designed a receptor-binding domain (RBD) nanoparticle vaccine. In mouse studies, SpFN induced potent nAb responses and protected against challenge.[28,29] In NHPs, SpFN coformulated with ALFQ elicited a potent nAb response against wild-type SARS-CoV-2 and the alpha and beta variants as well as a good response against SARS-CoV-1. Further characterization of the immune response showed a strongly biased T_H1 $CD4^+$ T cell response and an engaged memory response. SpFN also protected against viral replication in the lungs and airways after challenge.[30,31] SpFN is currently being investigated in a phase 1 study.[32,a]

COVID-19 vaccines: efforts on pan-coronavirus vaccines

The emergence of variants has already demonstrated that updated vaccines and/or boosting doses should be recommended to maintain high levels of immunity among the population. Several speakers discussed another approach: developing a pan-coronavirus vaccine that could protect against future variants and potentially against future pandemic strains.

Understanding how SARS-CoV-2 achieves genetic diversity

Ravindra K. Gupta from the University of Cambridge discussed how SARS-CoV-2 has achieved its genetic diversity. As discussed previously, the broad picture that is emerging indicates that current vaccines typically show a decrease over months in the nAb response to the alpha, beta, and delta variants. Currently, the real-world impact of this is that vaccinated individuals are at increased risk of breakthrough infection by variants (e.g., including by the recent Omicron variant), but protection against severe disease and death remains higher than without vaccination.[33–42]

Gupta described what is currently known about how SARS-CoV-2 mutates and gave an overview of the efficacy of current vaccines against several variants. Since SARS-CoV-2 emerged in late 2019, it has undergone significant increase in diversity. However, several features of SARS-CoV-2 suggest that there is relatively low selection pressure on the virus to evolve. The mutation rate is estimated to be relatively modest; it has a short incubation period and can be transmitted by asymptomatic individuals. Generally, viral mutations are a result of either antigenic drift (caused by errors during replication) or antigenic shift (caused by recombination of viral RNA within a host cell). Gupta noted that SARS-CoV-2 is changing this paradigm of viral evolution. There is currently limited evidence for recombination in humans.[b] Instead, SARS-CoV-2 appears to have achieved diversity via chronic infection. Gupta's group has tracked viral genetic diversity over time in individuals infected with SARS-CoV-2 and showed how different viral variants arise and decline within a given patient. Prolonged viral shedding in chronically infected patients can thereby be a source of new variants in the population at large.[43–45]

Efforts to develop a pan-coronavirus vaccine were recently published by Saunders *et al.* in which the authors developed a ferritin nanoparticle vaccine using a highly conserved spike protein epitope. The vaccine demonstrated neutralizing

[a]For a general overview post COVID of use of nanoparticles in the clinic, see Anselmo, A.C. and S. Mitragotri, 2021, https://doi.org/10.1002/btm2.10246.

[b]Emergence of the Omicron variant may, however, provide evidence for this, see https://osf.io/f7txy.

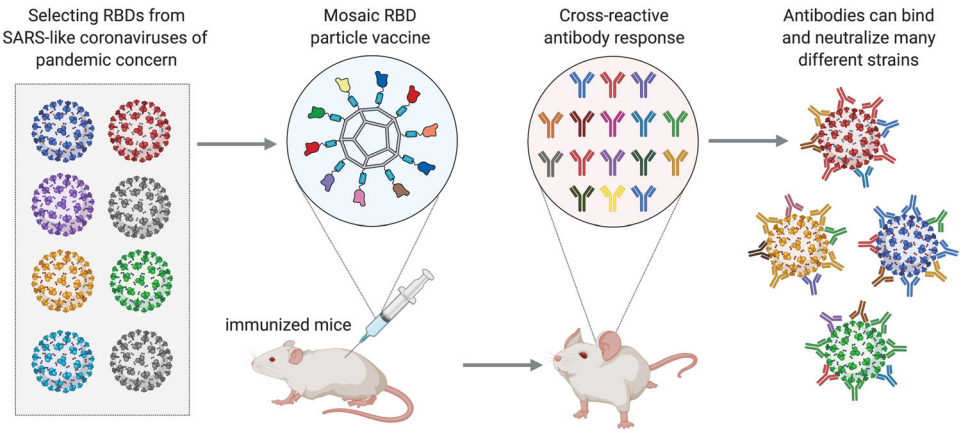

Figure 1. **Mosaic nanoparticle approach for making a potential pan-sarbecovirus vaccine.**

activity against wild-type and beta SARS-CoV-2, as well as against SARS-CoV-1 and other coronaviruses.[46] Gupta noted that this is a promising first step toward focusing more on the diversity of coronaviruses and a pan-coronovirus vaccine.

Structural insights on neutralization to inform the design of broadly active vaccines

Pamela J. Bjorkman from California Institute of Technology discussed work to identify structural correlates of neutralization to SARS-CoV-2, with the goal of informing a more broadly cross-reactive vaccine. Cryo-EM structures of approximately 30 nAbs from convalescent COVID-19 patients bound to the SARS-CoV-2 spike trimer revealed several common epitopes and binding modes. Bjorkman's group has classified these binding modes into four main groups based on whether the nAb blocks the interaction between the spike trimer and ACE2, which the virus uses to gain entry into the cell, and the position of the three receptor-binding domains (RBDs). Class 1 nAbs block ACE2-spike binding by binding to the spike trimer RBDs when they are in an up position. Class 2 nAbs block ACE2 binding and bind to the spike trimer when RBDs are in either the down or up position. Class 3 nAbs do not overlap with the ACE2 binding footprint and bind to the spike trimer when the RBDs are in an up or down position. Class 4 nAbs do not block ACE2 binding and bind to RBDs in an up position.[47,48] Additional studies have verified that antibodies elicited by mRNA vaccines are functionally similar to those induced by natural infection. Vaccine-induced antibodies are mostly Class 1 and Class 2, indicating that APCs present spike trimers that adopt RBD conformations similar to those observed on SARS-CoV-2 virions. However, Class 1 and Class 2 epitopes, which are located near the end of the RBD, are more variable and are the sites of mutation in SARS-CoV-2 variants.[49]

Bjorkman's group is using the insights gained from these antibody structures to develop a pan-sarbecovirus vaccine that protects against all SARS-like beta coronaviruses (Fig. 1). The hope is to elicit Class 3 and Class 4 antibodies that bind to the more conserved, though less accessible, regions of the RBD. They have developed a mosaic nanoparticle that incorporates RBDs from various SARS-like coronaviruses, including those with spillover potential from animal reservoirs. Bjorkman hypothesized that B cell receptors that bind with avidity to a conserved epitope present on multiple different antigens would be preferentially stimulated, potentially leading to the production of cross-reactive antibodies. Preclinical data suggest that mosaic nanoparticles have the potential to protect against current SARS-CoV-2 infection, against future variants, and potentially against emerging sarbecovirus strains. In mice, nanoparticles that codisplay SARS-CoV-2 RBD with RBDs from other viruses elicited similar anti-SARS-CoV-2 nAb responses as a homotypic SARS-CoV-2 nanoparticle. In addition, mosaic codisplay also achieved neutralization of strains included in the nanoparticle, as well as strains not included, suggesting that this approach can achieve broad protection against

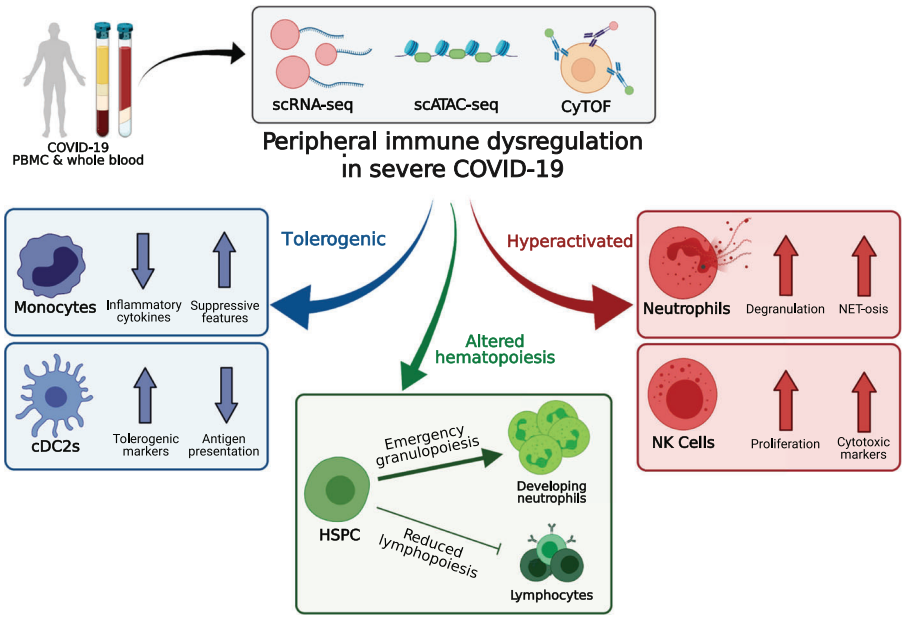

Figure 2. **Peripheral immune dysregulation in severe COVID-19 based on scRNA-seq, scATAC-seq, and CyTOF analyses to identify correlates of mild, moderate, and severe pathology. From Wilk *et al.* 2021, http://doi.org/10.1084/jem.20210582. Published under a Creative Commons Attribution License 4.0 (CC BY).**

sarbecoviruses, including those not represented in the vaccine itself.[50]

Integrated single-cell 'omics analyses on COVID-19 immune responses

Catherine A. Blish from Stanford University presented work using single-cell 'omics to inform pan-vaccine development strategies for SARS-CoV-2. Blish stressed the need to evaluate not only cases of severe COVID-19 disease but also of mild disease to understand what a good, effective immune response looks like and devise strategies to facilitate such a response. Blish's group recently showed via single-cell RNA sequencing (scRNA-seq) that patients with severe COVID-19 show a significant reconfiguration of monocyte populations.[51] In a more recent study, Blish's group conducted scRNA-seq, scATAC-seq, and CyTOF analyses to identify correlates of mild, moderate, and severe COVID-19 (Fig. 2).[52] Transcriptomics data from 33 COVID-19 patients across the disease spectrum revealed that neutrophil populations can accurately predict disease and disease severity. In particular, a population of developing neutrophils was enriched in patients with fatal disease. In addition, monocyte populations in uninfected controls and those with mild disease overlapped, potentially indicating that the absence of a monocyte-mediated inflammatory response may correlate with protection.[52] Using an integrated analysis of multimodal single-cell data, Blish's group achieved a more fine-grain analysis of lymphocytes subsets.[53] They showed that neutrophils, CD16 and CD14 monocytes, and $CD8^{+}$ T effector memory cells are among the most perturbed populations in COVID-19. In particular, monocytes have taken on a myeloid-derived suppressor cell–like phenotype in severe disease, a phenotype characterized by poor proinflammatory cytokine secretion. Transcription factor analyses showed that decreased NF-κB binding activity associates with disease severity, while scATAC-seq data showed changes in chromatic accessibility at the *IL1B* locus in severe and fatal disease. Together, these data indicate that aberrant decreases in NF-κB activity in severe COVD-19 may result in loss of accessibility at cytokine gene enhancers and subsequent decreased cytokine expression in peripheral monoctyes.[52] Previous studies have also shown that vaccination against influenza can reconfigure the epigenomic and transcriptional landscape.[54] Blish stressed that similar 'omics analyses of the effects of COVID-19

vaccination would be instrumental in understanding how vaccines prime the innate immune response. Blish ended her presentation with unpublished work on the role of adipose tissue in SARS-CoV-2 infection.

Toward a universal influenza virus vaccine—parallels for a pan-coronavirus vaccine

Florian Krammer from the Icahn School of Medicine at Mount Sinai presented work on developing a universal influenza vaccine. Influenza viruses are extremely diverse. Influenza A and B viruses are responsible for disease in humans and can be divided into several different subtypes based on the antigenic properties of their surface glycoproteins, most notably HA and neuraminidase (NA).[55] Krammer gave an overview of how the predominant influenzas virus subtypes have changed over time. Influenza virus has been responsible for several pandemics over the years, the H1N1 influenza pandemic in 1918, as well as an H2N2 pandemic in 1957 and an H3N2 pandemic in 1968. As populations develop immunity to a given strain, antigenic shift and drift of viruses enable new strains to take hold. Today, the primary circulating influenza viruses are H1N1, H3N2, and influenza B. This diversity, the ability to mutate, and the ability of influenza viruses to spill over from animal reservoirs make it difficult to develop a universal influenza vaccine. Such a vaccine would be instrumental in protecting against not only seasonal strains that experience antigenic drift throughout the year but also emerging pandemic strains.

Current influenza vaccines target the globular head domain of HA, which mediates binding to the host cell. While this region is an immunodominant antigen for influenza virus and elicits a strong nAb response, it is also the primary site of antigenic drift. In contrast, the HA stalk domain is more conserved across viral subtypes, thought it does not elicit as strong of an immune response.[56] Antibodies against the stalk domain are not significantly induced or boosted upon regular seasonal vaccination. However, they have demonstrated broad neutralizing activity that spans influenza subtypes and have been shown to independently correlate with protection from H1N1 infection in humans.[57,58]

Krammer's group is working on vaccination strategies to induce protective levels of broadly nAbs against the HA stalk domain. They have designed chimeric HA proteins composed of a head domain from one subtype and a stalk domain from another.[59] Sequential administration of vaccines that contain different head domains but the same stalk domain would be expected to elicit a primary immune response against the head domain after each dose, while generating a recall response against the stalk domain after each boosting dose. Preclinical studies showed that sequential immunization with chimeric HA proteins induced broadly reactive antistalk antibodies and protected animals from challenge with heterologous and/or heterosubtypic virus strains with good protection against emerging viruses.[60–65]

The ability of chimeric HA immunization to elicit broadly cross-reactive antibodies against the HA stalk domain was tested in a placebo-controlled, phase 1 clinical trial.[66] The study investigated different routes of administration of live, attenuated, and inactivated vaccines with and without an adjuvant. The adjuvanted inactivated vaccine induced strong, broad stalk-reactive IgG responses across group 1 viruses even after one dose.[66] Krammer noted that a truly universal influenza vaccine will require components from group 1, group 2, and influenza B viruses. A vaccine containing group 2 and influenza B constructs will be tested in clinical trials with group 1 vaccines. Krammer hopes that lessons learned from these studies can help to inform efforts to develop a pan-coronavirus vaccine to combat the diversity of coronaviruses present to date as well as new variants that emerge in the future.

Modeling impacts of COVID-19 vaccination strategies

Predicting how COVID-19 case rates will change is a complex problem that integrates disease control measures, vaccination strategies, immunology, epidemiology, and viral evolution.

Caroline E. Wagner from McGill University described work in collaboration with researchers at Princeton University to create a framework to model these concepts and project COVID-19 case rates based on different assumptions related to the strength and duration of natural and vaccine-mediated immune responses, vaccine dosing strategies, and vaccine sharing between regions.

Leveraging a model by Morris *et al.*,[67] Wagner and colleagues modeled several scenarios for the future trajectories of the magnitude and timing of COVID-19 cases based on different assumptions of the strength and duration of adaptive immune response following primary and secondary infections as well as vaccination.[68] They adapted a framework to project the epidemiological and evolutionary implications of vaccination, specifically looking at the effects of different spacings of the doses for two-dose vaccines. The model accounts for several immune categories, including one-dose and two-dose vaccine immunity, waned one-dose and two-dose vaccine immunity, and infection after one or two doses. They considered scenarios in which a single vaccine dose elicits robust immunity and one in which it elicits poor immunity. The model predicted that a strategy that focuses on vaccinating as many people as possible with their first dose is effective at curbing the size of the first epidemic peak after vaccination begins, but longer-term effects are less clear. If the first dose does not elicit robust immunity, this strategy may lead to more and larger peaks in infection as one-dose immunity wanes, compared with a strategy where both doses are given in more rapid succession. Further, while a one-dose strategy may increase the number of people immunized and reduce infections in the short term, in the longer term the recommended two doses should be given to mitigate the potential for antigenic evolution due to secondary infections in partially immune populations (i.e., those with waned natural or vaccinal immunity).[69]

To model how vaccination strategies may impact viral evolution, they considered that one or two vaccine doses and natural infections may have different effects on immunity and could, therefore, differentially contribute to viral evolution upon subsequent infection.[69] This draws on classical work by Grenfell *et al.*, which posits that viral replication rates and selection may be intricately linked within hosts. In individuals with low immunity, replication rates are high, but selection is expected to be low. On the other hand, in individuals with high immunity, replication rates are low, but selection is expected to be high. In individuals with intermediate immunity, there may be a sweet spot, wherein viral replication and selection are high enough to maximize the potential for viral adaptation.[70] Their results show that the burden and timing of COVID-19 infections and the potential for viral adaptation are shaped by immune responses following natural infection and one or two vaccine doses in the short and long term.[69]

Finally, Wagner and colleagues showed how different vaccine allocation schemes between regions may have epidemiological and evolutionary implications. The model considered two interacting countries, one with high access to vaccines and one with low access that receives a specific fraction of vaccines from the other region. They considered scenarios in which individuals do (coupled) or do not (decoupled) move between the two regions, and in which the potential for viral adaptation may result in global transmission increases, simplistically simulating viral evolution (coupled). In both the decoupled and coupled frameworks and across a range of immunological scenarios, they found that sharing vaccines was effective for decreasing the potential for viral evolution. In the decoupled framework for symmetric country characteristics, vaccine sharing also uniformly maintained or reduced the equilibrium infection burden. In both frameworks, the burden of Covid-19 under a given vaccine sharing scheme was found to be sensitive to the global vaccination rate, the strength and duration of natural and vaccinal immunity, and asymmetries between countries (i.e., in terms of population size and national transmission rates) (Fig. 3). These results emphasize the importance of rapid and equitable vaccine distribution.[71]

Pandemic preparedness: lessons from COVID-19

Identifying correlates of protection in COVID-19 vaccine phase 3 trials

Lawrence Corey and **Peter B. Gilbert** from Fred Hutchinson Cancer Research Center described an approach to identifying correlates of protection (CoPs) in phase 3 efficacy trials conducted under the U.S. government Operation Warp Speed program. Designing the trials for standardized evaluation of CoPs was an important aspect of harmonizing the clinical development process to generate the evidence required for appropriate use of CoPs for predicting vaccine efficacy. Toward that goal, the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) public–private partnership harmonized the clinical trial designs so that they reported common efficacy endpoints and worked

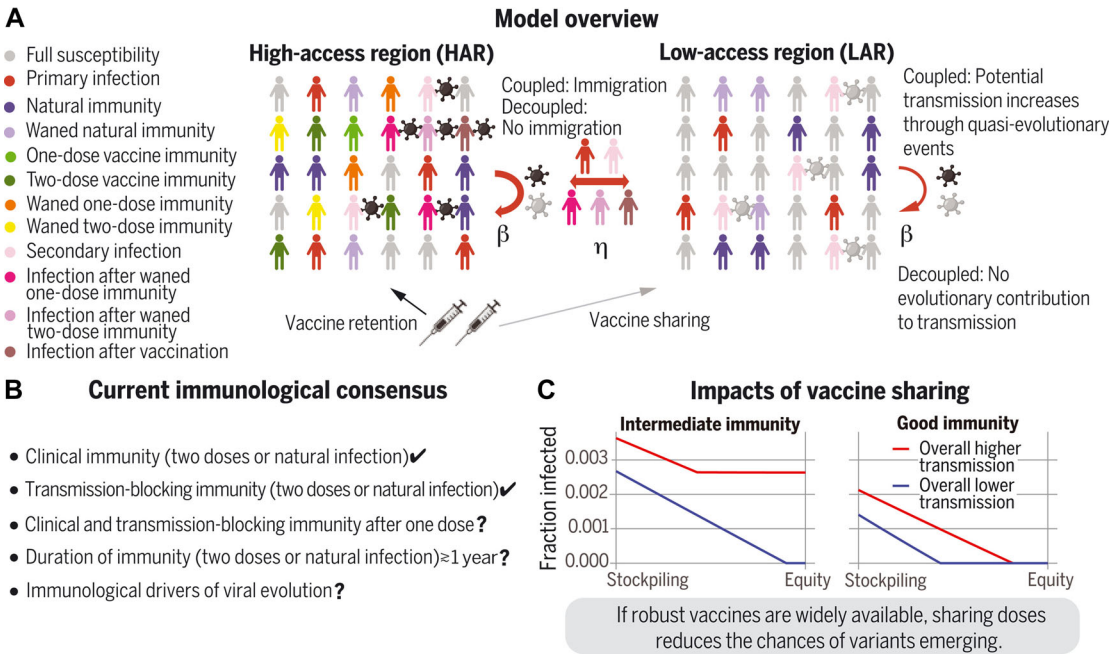

Figure 3. Effects of vaccine allocation on the epidemiological and evolutionary trajectories of two regions. (A) Each region is described by an immunoepidemiological model, and the regions are potentially coupled through immigration and transmission increases driven by viral evolution. (B) Current consensus on host immune responses and clinical and transmission-blocking protection. (C) Epidemiological and evolutionary outcomes for different vaccine allocation schemes given the specific assumptions related to natural and vaccinal immunity described in B. The terms "ointermediate" and "ogood" immunity in C follow the descriptions provided in the main text. Additional scenarios can be explored using the online interactive application (https://grenfelllab.shinyapps.io/vaccine-nationalism/). Schematics of needles and viruses in A were created with BioRender.com. From Wagner *et al.* 2021, https://doi.org/10.1126/science.abj7364. Published under a Creative Commons Attribution License 4.0 (CC BY).

with collaborating laboratories to define COVID-19 infection post vaccination and quantify immune responses. These harmonization steps enable standardized within—and between—trial data analyses to identify CoPs.[72]

Originally, it was hoped that a mechanistic correlate of protection could be identified early in the clinical development process that could be used to infer vaccine efficacy across different platforms so that CoPs could be used as endpoints in subsequent trials, precluding the need for a placebo group. Data analyses are underway to determine how well CoPs apply across vaccine platforms; it remains an open question how well they will apply; however, it should be recognized that it is more challenging to establish that a CoP can be used across vaccines than it is to establish use of a CoP for a given vaccine. While they have not been used to infer efficacy across vaccine platforms, they have been instrumental in inferring efficacy between different populations within a vaccine type. For example, for clinical trials in children and teenagers, immunogenicity correlates are major components of regulatory approval based on the larger placebo-controlled phase 3 trials in adults. Efficacy studies of monoclonal antibodies for treatment and prevention suggest that neutralization titers are a mechanistic CoP. Current evidence supports the conclusion that neutralization titer can operate as a CoP across variants, with the amount of decrease in neutralization predictive of the amount of decrease in vaccine efficacy (e.g., see Comer *et al.* 2021. https://doi.org/10.1016/S2666-5247(21)00267-6). Yet, much more validation of this model is needed, and it is likely that other immune responses will be required to fully explain how protection varies across variants.

Gilbert described how the statistical group at the COVID-19 Prevention Network (CoVPN) has been

analyzing data from phase 3 trials to do a consistent analysis for correlates of risk (i.e., how well antibody markers correlate with acquisition of disease) and CoPs (how vaccine efficacy varies with the level of antibody markers). They are also looking at how these correlates are affected by population demographics, prior infection status, time since vaccination, and virus genotype/phenotype. Correlates of protection have been instrumental in HIV-1 vaccine trials; these analyses have shown that T cell markers are predictive of HIV-1 acquisition and that combination markers of T cell and antibody characteristics can provide improved predictive value.[73–76] With regard to COVID-19, a recent study of the ChAdOx1 nCoV-19 vaccine suggests that live virus neutralization assay may be a strong correlate of efficacy.[77]

Several questions remain regarding CoPs to COVID-19 vaccines, including whether CoPs defined earlier in the pandemic when there was little strain variation will be applicable to current and future variants. In addition, cellular immunity was not systematically analyzed in the phase 3 clinical trials; it may be difficult to assess the impact of cell-mediated immunity on protection.

The impact of COVID-19 on the regulatory landscape

Gordon Dougan from the University of Cambridge discussed the impact of the development of COVID-19 vaccines on the regulatory process and how regulation can be improved in the future to improve vaccine equity. National and regional regulatory authorities are critical for ensuring that vaccines are protective and efficacious and reach international standards of quality and safety. Dougan stressed the importance that all countries have an effective national regulatory authority (NRA) that is independent of political and commercial interference and has enforcement power to protect the populations they serve, for example, by removing vaccines from the market if they do not meet certain standards. While many Western countries have strong NRAs, such as the FDA and the EMA, many national NRAs are essentially nonfunctional. Lack of vaccine regulation leaves populations vulnerable to exploitation through poor quality vaccines, vaccines designed for other regions where the variants differ, or unaffordable vaccines. To compensate for the lack of regional regulation, the WHO has established the Strategic Advisory Group of Experts on Immunization (SAGE), which advises the WHO on aspects of vaccine development, manufacturing, and usage, and sets a minimum international standard of quality for a vaccine through prequalification.

As several speakers described, COVID-19 vaccines were developed so quickly because of public financial input that enabled pharmaceutical companies to conduct several steps of the clinical development process in parallel, as opposed to sequentially. However, regulatory authorities also approved vaccine candidates in record time. Many countries used their emergency regulation review processes that allows vaccine candidates to be approved on a provisionary basis as more data are collected. In addition, the fact that several vaccines using multiple platforms showed such high efficacy made it easier to weigh the benefit-to-risk ratio of vaccination. Dougan also described the Medicines and Healthcare products Regulatory Agency's (MHRA) vaccine review process in the UK, which included several groups of internal and external experts as well as ongoing conversations with other international regulatory bodies. Finally, many preclinical datasets typically included in a candidate vaccine dossier were eliminated or merged as the emergence of clinical data rendered them unnecessary.

Dougan ended with thoughts on how vaccine equity can be improved by using systems within LMICs to develop vaccines, as opposed to going through Western development and regulatory processes, which can be timely and expensive. Typhoid conjugates, oral cholera, and rotavirus vaccines have already been taken through this route. However, expanding this to more vaccines and to other regions, such as Africa, will require expanding and establishing the global regulatory structure as well as regional manufacturing and regulation.

Toward equitable vaccine manufacturing, distribution, and roll-out

Nicole Lurie from the Coalition for Epidemic Preparedness Innovations (CEPI) discussed the organization's efforts to ensure sufficient manufacturing capacity and equitable distribution of COVID-19 vaccines. CEPI was established in 2017 to develop vaccines for viruses with epidemic potential. In January 2020, when SARS-CoV-2 appeared on the global stage, CEPI was involved in developing a

vaccine for another coronavirus, MERS. They shifted vaccine development efforts to focus on the emerging pandemic, but it quickly became clear that there was no global system to finance or facilitate an end-to-end vaccine response, from vaccine manufacturing and procurement to distribution. One of the issues with not having a global financing system for vaccine development is that there were no entities that could make at-risk investments for LMIC. High-income countries like the United States and UK made at-risk investments for vaccine development and at-risk commitments to purchase vaccines. This allowed countries to make bilateral deals with pharmaceutical companies to ensure sufficient supply for their country but left many LMICs out of the loop. To address this gap, CEPI cofounded COVAX in partnership with Gavi and the WHO, with an aim to accelerate the development and manufacturing of COVID-19 vaccines while ensuring fair and equitable access around the world.

In addition to establishing processes for global vaccine manufacturing and distribution, it is also key to ensure that there are enough resources and materials to manufacture vaccines. An estimated 10–14 billion doses of COVID-19 vaccines will be required, on top of the 4–5 billion non-COVID-19 vaccine doses that are typically manufactured annually. As manufacturing of COVID-19 vaccines ramped up, raw materials became scarce. Lurie stressed that suppliers need to have a commitment from buyers to invest in increasing their manufacturing capacity. In the case of COVID-19 vaccines, that demand signal came too late for suppliers to ramp up production to prevent acute shortages. In addition, export and custom regulations between countries can slow down the shipment of goods and affect the entire supply chain. To address these problems, CEPI has reserved manufacturing capacity at a network of facilities around the world and secured raw materials as a hedge against any one country buying up supplies to ensure equitable access. They have also engaged the World Trade Organization to facilitate the free flow of goods around the world and set up a confidential marketplace to bring together vaccine manufacturers and raw material manufacturers. Lurie stressed that while these systems were put in place to address COVID-19, they are designed to endure to address future pandemics and supply chain issues.

Panel discussion

The session ended with a panel discussion with **Corey**, **Dougan**, **Gilbert**, and **Lurie** moderated by **Shabir A. Madhi** and **Christopher L. Karp** about how to improve vaccine equity. Dougan noted that addressing ongoing pandemics that primarily affect LMICs, such as tuberculosis, HIV, and malaria, will require participation not only from large coalitions and funders like COVAX and the Gates Foundation, but also country and community participation. In resource-limited settings, efforts often do not endure because of lack of support from local communities.

Lurie stressed that more equitable distribution will require improving regional regulatory systems and laboratories to establish regional manufacturing capacity, which is virtually nonexistent in places like Africa. Without a strong regulatory system, the need to develop vaccines quickly, to identify correlates of response, and inherent vaccine nationalism can lead to vaccines being authorized with a subpar level of evidence. Although there is currently an acute need to establish stronger systems in LMICs, these gaps require long-term solutions that include establishing an educated skilled workforce. At its core, this consists of building a foundation via sustainable models for science education and research and development as well as addressing the brain drain of educated individuals to wealthier countries. To begin to address this, CEPI has engaged investigators in LMIC countries to collect epidemiologic data and conduct laboratory analyses to increase their capacity to conduct clinical trials. In addition to building out the research and development capacity, there is also a need to increase manufacturing capacity in LMICs. Lurie noted that for manufacturing capacity to endure, there must be a sustainable business case for manufacturers. Even if mRNA vaccines prove to be an enduring technology for future pandemics, exemplified by the flexibility and high efficacy seen with COVID-19 vaccines, transferring that technology to regions like Africa may not be feasible to stop the spread of COVID-19. However, the panelists are hopeful that advances in tuberculosis and HIV vaccines can provide a sustainable market to make manufacturing worthwhile for the private sector.

When asked how the next pandemic can be better addressed, Dougan noted how COVID-19

revealed the value of a global surveillance system to both track disease and monitor threats. The systems developed during COVID-19 may facilitate earlier identification of novel threats. Gilbert stressed the need to characterize breakthrough cases to validate CoPs and ultimately use viral genotype to predict how different vaccines will work in different settings. Corey noted that the pandemic response could have been improved by repurposing academia more efficiently, particularly early in the pandemic when diagnostic tests were slow to be developed. In addition, funding for a concerted strategy and public–private partnerships proved to be so instrumental in making vaccines available. In the future, having a more global perspective early on can ensure that these types of collaborations benefit the world and not just wealthier countries. Lurie also stressed the need for processes and global accountability for developing diagnostics, vaccines, and therapeutics, with adequate regional funding and the capacity for surge funding to facilitate manufacturing when these products are available.

Identifying correlates of immune protection

Understanding the humoral immune response to vaccines and the immune correlates that mediate protection against disease is particularly critical in this new age of shortened vaccine development timelines. Established CoPs have the potential to be used as surrogate endpoints in clinical trials to determine the efficacy of vaccine candidates without the need for large, time-consuming placebo-controlled trials.

Systematic approaches to identifying immune correlates of response in COVID-19

Galit Alter from MIT and Harvard University is developing tools to holistically and objectively profile the types of antibody-mediated immune responses to understand which mechanisms are most relevant to protection and help to guide vaccine development. Using systems serology and a number of parallelized assays linked to machine learning developed in collaboration with the Gates Foundation, Alter described how they can profile the biophysical and functional characteristics of antibodies, such as the overall levels of different antibody subclasses and isotypes specific for numerous epitopes, antigen-specific antibody binding to Fc receptors, post-translational modifications of antibodies, and the ability of antibodies to recruit effects by complement, neutrophils, dendritic cells, macrophages, adaptive immune cells, and other immunological functions. The system integrates all this information and uses machine learning to identify patterns associated with clinical outcomes to essentially understand the biophysical and functional features of antibodies associated with disease control.

Alter's group has used this approach to understand the immune response to SARS-CoV-2 infection and COVID-19 vaccines. COVID-19 vaccines have shown impressive protection across various platforms. As viral variants have emerged, there has been concern over whether the vaccines will continue to provide effective protection. Reports from J&J, Moderna, and Pfizer have indicated that while vaccines show loss of nAb activity against the variants, they continue to protect against severe disease. While nAbs that bind to a pathogen and block infection through neutralization are typically regarded to be key to immunity, antibodies are involved in many other functions. The majority of antibodies survey tissues and the periphery for pathogens or pathogen components on infected cells and act as recruiters of other immune effectors. Current data on SARS-CoV-2 variants suggest that factors other than neutralization may be instrumental for protection. Understanding these factors could help when designing next-generation COVID-19 vaccines and to strategically boost it when it starts to wane.

Applying a systems serology approach to patients with COVID-19 identified distinct patterns of antibody responses among patients who survived and those who died. Survivors had early high titers of spike-IgG antibodies, while nonsurvivors had a delayed spike IgG response. Alter showed that during natural infection, these antibodies drive opsinophagocytic clearance by monocytes or complement, which may be critical for the early control needed to overcome disease. They also looked at the role of adjuvants in a SARS-CoV-2 subunit vaccine in NHPs. They administered the vaccine with five different adjuvants and monitored the antibody profile. Alter showed that while all five adjuvants induced IgM, IgG1, and IgA responses, some induced higher titers than others. Adjuvants also

shaped antibody function. While the priming dose was sufficient to induce antibody titers, a boosting dose was required to induce humoral immune response functions, such as NK cell activity, neutrophil phagocytosis, and complement activity. To understand which of these functions matters most to protective immunity, they challenged the animals with virus. They found that while neutralization correlated with viral control, it was not a strong predictor of protection. Instead, Fc function was found to discriminate between protected and non-protected animals.[78] Alter concluded by saying that non-neutralizing Fc-effector functions, specifically opsinophagocytosis, are likely key to both natural resolution of SARS-CoV-2 infection and vaccine protection.

Immune correlates of protection from tuberculosis

Elisa Nemes from the University of Cape Town discussed ongoing studies to identify immune correlates of protection for the tuberculosis vaccine BCG. While BCG has been available as a vaccine for tuberculosis for nearly 100 years, it has not stopped the epidemic. Much of the world has universal BCG vaccination recommendations, but there are still 10 million new cases and 1.4 million deaths each year.[79] It is clear that new, more effective tuberculosis vaccines are needed; but vaccine development has been slow, despite the fact that there are several tuberculosis vaccines in the development pipeline.[80–82]

Two key developments in 2018 provided the first opportunity to discover immune correlates of protection against tuberculosis in humans. First, revaccination of BCG was shown to have approximately 45% efficacy in protecting uninfected individuals from acquiring established *M. tuberculosis* infection.[83] Second, the novel vaccine M72:AS01E showed approximately 50% efficacy in preventing tuberculosis disease in infected adults.[84,85] Both strategies show strong signs of immunogenicity. BCG revaccination significantly increased T_H1 and IL-22–producing $CD4^+$ T cells.[86] M72:AS01E elicited strong, durable antibody titers and a potent, polyfunctional T_H1 response.[85] However, immunogenicity is not always indicative of protection. Therefore, the Bill & Melinda Gates Medical Research Institute is leading a global effort to identify CoPs from these trials. While this is the first large-scale efforts to identify CoPs in humans, some clues are available from NHP studies. For example, lung immune responses to BCG, including antigen-specific T_H1, IL-17, and IL-10, correlated with protection against infection and disease in rhesus macaques.[87] Additional CoPs are being defined using the intravenous BCG NHP model.[88]

B cell responses to yellow fever vaccine

John Tyler Sandberg from Hans-Gustaf Ljunggren's group at Karolinska Institutet presented work on the humoral response of the yellow fever vaccine YFV 17D, a live, attenuated viral vaccine, developed over 70 years ago, that provides strong, lifelong immunity against yellow fever. It is often used as a model vaccine for immunologic studies, as well as a model virus infection to characterize human immune responses. Previous work in Ljunggren's lab characterized the T cell[89] and NK cell[90] responses to vaccination. In a study of 24 healthy volunteers who received YFV 17D, vaccination increased germinal center activity followed by an increase in circulating T_H1-polarized circulating T follicular helper cells. Vaccination also induced antigen-specific plasmablasts within 2 weeks and elicited nAbs and antigen-specific memory B cells (Fig. 4). Sandberg's work has added to the understanding of YFV 17D on the adaptive immune response and the events leading to B cell immunity.[91]

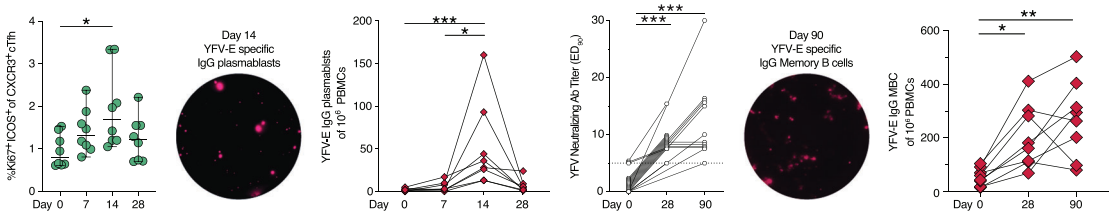

Figure 4. **YFV-17D vaccination also induced antigen-specific plasmablasts within 2 weeks and elicited nAbs and antigen-specific memory B cells. Adapted from Sandberg *et al.* 2021, https://doi.org/10.4049/jimmunol.2001381.**

Improving pediatric responses to vaccination

Kiva Brennan from Sarah Doyle's lab at Trinity College Dublin presented work on cytosolic dsRNA to improve the innate immune response to pediatric vaccines. Pediatric vaccine schedules often require multiple boosters of the same vaccine or are given later in life to achieve a good immune response. This results in a window of vulnerability during which children receive vaccines but are still susceptible to disease. Part of the explanation for this is the immune system of children is different than that of adults.

Brennan is investigating effective pediatric adjuvants that can boost vaccine efficacy. They showed that cytosolic poly(I:C) (cPIC), a double-stranded RNA that activates the RIG-I/MDA6 pathway, induces an IFN response in neonatal cells. In contrast, TLR4 stimulation, which induces an IFN in adult cells, does not induce the same response in neonatal cells. Neonatal monocytes show decreased expression of Rab11, an important protein involved in endosome formation. Brennan showed that the TLR-induced IFN relies on endosome formation, whereas cPIC does not.[92] Brennan showed unpublished work how combinations of cPIC and currently used adjuvants (e.g., alum or MPLA) impact the innate immune response.

The potential for vaccines to shift paradigms in global health

Reducing antimicrobial resistance with vaccines

Elizabeth J. Klemm from the Wellcome Trust discussed the role of vaccines in combatting antimicrobial resistance (AMR). AMR is a significant problem, contributing to approximately 700,000 deaths annually, a number expected to rise to 10 million by 2050.[93] Tackling AMR will require a multifaceted strategy, of which vaccines are one tool. There are several ways in which vaccines can impact AMR. First, they can directly reduce infection, carriage, and transmission of drug-resistant pathogens, as well as prevent secondary infections with drug-resistant pathogens. For example, introduction of the pneumococcal conjugate vaccine (PCV) led to a decrease in rates of invasive pneumococcal disease caused by drug-resistant strains.[94,95] Vaccines can also reduce antibiotic use, both for the pathogen that they protect against and for other pathogens by reducing the occurrence of secondary infection and reducing empiric antibiotic use. For example, in low- and middle-income countries, children vaccinated with PCV had lower odds of antibiotic treatment for acute respiratory infections, resulting in an estimated 24 million fewer antibiotic-treated episodes per year.[96] In an observational study in Canada, regions with universal influenza vaccine access had lower numbers of antibiotic prescriptions.[97] Similarly, a typhoid vaccine would likely reduce empiric antibiotic use for typhoid-related symptoms, which is typically viral in nature. It is currently estimated that 3–25 patients are unnecessarily given antibiotics for every true case of typhoid.[98] Two systematic reviews recently reviewed the evidence on vaccines and antibiotic use. While the authors concluded that pneumococcal and influenza vaccines likely reduce antibiotic use, they also pointed to several data gaps in the field. Differences in study design, data reported, vaccines used, and populations tested make it difficult to draw broad conclusions, and there are little data from LMICs or for vaccines other than those against pneumococcal disease and influenza. To fill these knowledge gaps and expand the evidence base for the effect of vaccines on a range of pathogens, the Wellcome Trust has recently funded several ongoing projects throughout the world.

Klemm also discussed the Wellcome Trust's efforts to identify priority pathogens for the development of vaccines to reduce AMR. While the WHO and CDC have identified priority pathogens for development of antibiotics, there is no corresponding list for impacting AMR. In 2018, the Wellcome Trust published an analysis of the WHO's list of priority pathogens on suitability for vaccine development, assessing factors including health impact, R&D feasibility, and probability of uptake to provide actionable recommendations for funders and vaccine developers (the report is available online at VaccinesforAMR.org). The report grouped pathogens based on the aforementioned metrics and provided recommendations for each group. For example, for pathogens for which vaccines already exist, such as *H. influenzae, S. pneumoniae,* and *S. typhi,* efforts should focus on increasing vaccine uptake and access. For pathogens with preclinical data, such as *E. coli* (enteric), non-typhoidal *Salmonella,* and *Shigella,* recommendations include focusing on accelerating clinical development to bring new vaccines to marker. High-impact

pathogens for which R&D feasibility is unclear and investments in early stage research are warranted include *M. tuberculosis, N. gonorrhoeae*, *E. coli* (urinary), *P. aeruginosa*, and *S. aureus.* Finally, for pathogens that are less well suited for vaccine development, including *S. paratyphi, Campylobacter*, *H. pylori, K. pneumoniae*, and *A. baumanii*, the report recommends exploring alternative strategies.

In 2021, the WHO published an action framework to leverage vaccines for preventing AMR. The framework describes three goals: expand the use of licensed vaccines; develop new vaccines that contribute to prevention and control of AMR; and address funding, regulatory approval, and technical challenges. Klemm stressed that achieving these goals will require new approaches to routinely collecting and analyzing data on AMR in vaccine trials in a harmonized way that enables cross-study comparisons. In addition, studies on the economic impact of vaccines on AMR are vital for decision making and modeling the cost-effectiveness of vaccines. While such data are limited, a recent report in China estimated that expanding coverage of the pneumococcal vaccine could lead to $586 million dollars in savings over 5 years.[99]

Addressing challenges for implementing vaccination strategies

Ankur Mutreja from the University of Cambridge discussed some of the logistical challenges in implementing infectious disease control measures, including vaccination strategies in communities.

One of the first challenges in implementing a vaccine strategy is identifying the problem. Communities may be reluctant to accept that there may be a problem, and cultural and religious issues may factor into an unwillingness for communities to trust outsiders. In addition, researchers looking to set up surveillance can run across difficulties navigating local approval processes, customs, and regulations and may not have the infrastructure necessary to apply monitoring tools in a field setting. Mutreja pointed to Tanzania as an example, which, early on, reported essentially zero COVID-19 cases. Given the rates of infection in surrounding countries, it is likely that the true prevalence of infection was masked due to a gap in surveillance and a lack of customized tools.

Once the problem has been identified, the next challenge is identifying the cause. This is often complicated by difficulties teasing out associations from causes and the involvement of multiple causes. Again, this requires trust from the local community to understand the complexities of the issue as well as the ability to revise methodological approaches as new information comes to light. This may not always be feasible depending on the prior approvals obtained by local authorities.

Once the problem is reasonably understood, solutions can be developed and executed. Mutreja stressed that there is often a difference between how the solution is planned and how it is executed, once again highlighting the importance of flexibility and improvisation. Challenges to executing plans include understanding the local and regional governments and authorities. For example, plans to intensify India's efforts to vaccinate 90% of infants were initially made with the country's Ministries of Health and Women and Child Development. Ultimately, 12 additional nonhealth ministries were included in implementing the plan. While the efforts increased full immunization coverage by 18%, they failed to meet the 90% goal.[100] Other hurdles include lack of healthcare data or regulatory infrastructure as well as logistical problems with storage and distribution. Finally, recruiting a local skilled workforce and generating buy-in from the local community are essential. Several of these challenges are apparent in the current inequities in COVID-19 vaccine distribution. A recent study showed that while only 16% of the world's population is in high-income countries, 65% of COVID-19 vaccine orders come from those countries.[101]

One of the most important steps is measuring the impact of an intervention. This both justifies the relevance of the intervention and helps to build trust with the community. However, lack of robust data recording systems, a skilled workforce, and continuous participation can complicate this. In addition, it can sometimes be difficult to know what indirect impacts to measure. For example, high Ebola disease burden in Sierra Leone decreased essential healthcare and demand for essential healthcare services. As a result, health outcomes across the board were affected, including increased maternal mortality, excess mortality from HIV, tuberculosis, and malaria, and an increase in non-Ebola outbreaks. An effective monitoring strategy must take into account all the impacts of the intervention.

Finally, sustaining an intervention's effects can be difficult due to changing political environments and a lack of long-term funding. It often requires recruiting a training to local skilled workforce and establishing robust data flow systems to ensure that monitoring continues. As with every step of the process, establishing trust with the community is key to ensuring long-term success.

Understanding differential outcomes of oral vaccines

Gagandeep Kang from Christian Medical College and the Translational Health Science and Technology Institute presented results from the Rotavirus Vaccine Immunogenicity Study to better understand why vaccines do worse in low-income settings compared to high-income settings. A systematic review of commercial rotavirus vaccines showed that postimmunization antirotavirus IgA levels were detectable in ∼90% of individuals in high-income countries but only 53% of individuals in low-income countries.[102] Several possible explanations have been proposed to explain the difference, including factors that impact a child's inoculum, such as transplacental and breast milk maternal antibodies, and factors that impact the antibody response, including coadministration of other vaccines, nutritional deficiency, the microbiome, and early exposure to other pathogens. Kang has been involved in multiple clinical trials to understand how and whether these factors impact immunogenicity.

Kang described the results of the Rotavirus Vaccine Immunogenicity Study[103] conducted in the UK, Malawi, and India to investigate the impact of maternally derived antibodies and infant microbiota on the immunogenicity of oral rotavirus vaccines in infants. Kang described that rates of viral shedding and seroconversion were lower in India and Malawi than in the UK. One unique factor in Indian children was neonatal rotavirus exposure prior to vaccination in approximately half of infants. Up to one-third of Indian infants were seropositive prior to vaccination due to neonatal infections. Prior infection was associated with a lower likelihood of viral shedding post vaccination but did not impact seroconversion. Kang showed that high levels of maternal antirotavirus IgG antibodies were associated with reduced vaccine response in children. A more detailed analysis of the Indian cohort showed that maternal IgG and IgA antibodies in serum and breast milk did not prevent neonatal rotavirus infection and decreased infant response to vaccine. Neonatal rotavirus infection prior to vaccination was associated with a higher response to vaccination. While differences in inflammatory biomarkers were observed across the three countries, none were associated with seroconversion or shedding. There were also geographic differences in microbiome composition and diversity, with microbiota diversity negatively correlating with vaccine response.[104]

The study supports several recommendations for future research, such as including shedding as a measure of response to oral rotavirus vaccines, considering a neonatal dose in lower income countries, and profiling early life host–microbe interactions in low-income settings.

Reverse vaccinology to develop an invariant Trypanosoma vivax *antigen-based vaccine*

Gavin J. Wright from the Wellcome Sanger Institute and University of York discussed work on developing a vaccine for *T. vivax*. Trypanosome parasites (Fig. 5) cause significant impact on important livestock animals in Africa, killing approximately 3

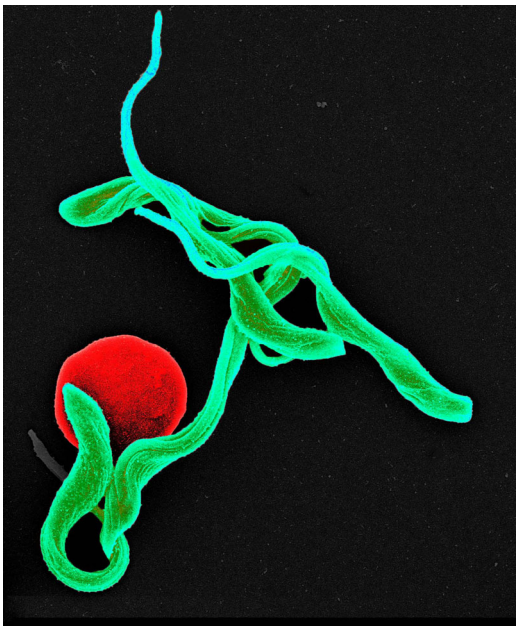

Figure 5. ***Trypanosoma vivax*. Credit: David Goulding, Wellcome Sanger Institute.**

million cattle each year and causing a direct economic cost estimated at $650 million. The effect is so severe that the Food and Agricultural Organisation of the United Nations stated that animal African trypanosomiasis "lies at the heart of poverty in Africa." The most common species that affect livestock are *T. congolense* and *T. vivax.* While infection is currently managed with drugs, they can cause side effects, and resistance is emerging.

Trypanosomes have adopted several strategies to evade the host immune system. First, parasites use antigenic variation, in which they change the expression of the surface antigen variable surface glycoprotein (VSG) throughout the course of infection. As the host develops antibodies against a particular VSG variant, the parasite expresses a new VSG, rendering it invisible to the current antibody response. This results in waves of parasitemia that eventually lead to chronic infection.[105] Trypanosomes also thwart antibody-mediated responses by endocytosing host antibodies.[106] Both features have made it difficult to develop trypanosome vaccines. Previous efforts with inactivated/attenuated parasites and recombinant proteins have resulted in partial protection.

With the recent availability of the trypanosome genome, Wright's group is taking a reverse vaccinology approach to identify an invariant protein suitable for a subunit vaccine. From the *T. vivax* genome, Wright's group identified cell surface and secreted proteins expected to project beyond the VSG coat to generate a library of recombinant *T. vivax* vaccine candidates. They expressed the extracellular portions of these proteins in mammalian cells to retain structurally important disulfide bonds and glycosylation patterns and assessed them in a murine infection assay in which infection can be tracked *in vivo* via live imaging. Of the approximately 40 antigens tested, one, V23, elicited protective immunity in the murine model.[107]

V23 is a Type 1 surface protein with no known protein domains or paralogs within *T. vivax* or orthologs in other *Trypanosoma* species. Wright showed that V23 is located at the flagellar membrane, which is involved in antibody endocytosis. V23 primarily mediates immunity via antibodies, as anti-V23 antibodies can passively transfer protection, though there was no obvious correlation between antibody protective efficacy and epitope location or binding affinity. Given that mouse IgG1 antibodies do not potently recruit immune effectors, Wright's group switched the isotype of an anti-V23 monoclonal antibody to IgG2a, which can recruit immune effectors, and showed that this isotype-switched antibody elicits strong protection. Individually mutating the immune effector recruitment sites in the Fc region of anti-V23 IgG2a revealed multiple mechanisms of immunity, including significant involvement of the complement system.[107] Wright's work shows that it may be possible to develop protective vaccines against trypanosomes that could have significant impact on the socioeconomic development in Africa.

Outer membrane vesicles as vaccines and delivery system

Mariagrazia Pizza from GlaxoSmithKline discussed the use of outer membrane vesicles (OMVs) as both antigens and delivery system for heterologous antigens. OMVs are naturally produced by Gram-negative bacteria and contain many of the bacterial outer membrane proteins, which are often highly immunogenic antigens.[108] OMVs have been used to fight outbreaks in several countries and shown to be very efficacious.[109,110] Several induction methods have been developed to promote hyperproduction of OMVs; these often rely on chemical treatments or physical manipulations that disrupt the cell membrane and may alter OMV composition and physical properties.[108] Pizza described GSK's efforts to engineer bacteria that naturally hyperproduce OMVs.[111,112] The resulting OMVs, dubbed generalized membrane module antigens (GMMAs), are naturally released by bacterial strains engineered to hyper-bleb and are exclusively composed of outer membrane and periplasmic antigens. Additional features can also be included by engineering the strains with 1 mutations that detoxify LPS, delete undesired antigens, or overexpress heterologous antigens, resulting in vesicles with characteristics of safe and immunogenic vaccines.

Pizza presented examples of GMMA-based vaccines developed against *Shigella* and nontyphoidal *Salmonella.* A GMMA vaccine developed from a *Shigella sonnei* strain genetically modified to reduce the endotoxicity of LPS, while retaining the immunodominant O-antigen component of LPS, showed significantly reduced reactogenicity and

high immunogenicity in animal models.[112,113] In clinical trials, this GMMA vaccine was tolerated in both naive and exposed adults and showed dose-dependent immunogenicity.[114,115] GSK is working to develop a new *S. sonnei* strain that expresses higher amounts of O-antigen for future clinical trials. A similar strategy has been used for *S. typhimurium* and *S. enteritidis,* in which GMMA was used as a carrier for O-antigen. In mice, immunization with GMMA induced a similar IgG response as immunization with the O-antigen glycoconjugate, while inducing higher levels of functional antibodies.[116]

Pizza also showed how GMMA can be used as a platform to present not only endogenous antigens but also heterologous antigens that bacteria are engineered to overexpress, as well as chemically conjugated polysaccharides or protein antigens. *Neisseria meningitidis* serogroup B (MenB) GMMA conjugated to MenC or *Hemophilus influenzae* type b (HiB) polysaccharide antigens induced functional antibodies after a single dose. MenB GMMA conjugated to MenA or MenC polysaccharides induced higher titers than traditional carrier protein conjugate, suggesting that GMMAs may be able to present antigens in a more natural form. Protein antigens conjugated to GMMAs also showed high immunogenicity. In addition, GMMAs offer an attractive carrier for multiple antigens for multivalent vaccines.[117]

GMMAs have been studied as both antigens themselves and as a delivery system that show increased immunogenicity compared to classical conjugates or recombinant antigens. Pizza stressed that GMMAs offer a simple and cost-effective platform technology that is amenable to developing vaccines for LMICs.

Sowmya Ajay Castro from Helge Dorfmueller's group at the University of Dundee described work to develop vaccine candidates for Group A *Streptococcus* (GAS). GAS, also known as *S. pyogenes*, is a human-exclusive Gram-positive bacterium responsible for a wide range of infections. Initial episodes of GAS infection can induce pharyngitis, impetigo, and scarlet fever, while longer-term episodes can lead to life-threatening complications, such as acute rheumatic fever, necrotizing fasciitis, streptococcal toxic shock syndrome, and immune-related sequelae.[118] Although GAS remains sensitive to penicillin globally, recent studies have highlighted antibiotic resistance in patients with both pharyngitis and tonsillitis.[119,120] A promising approach to lessen the burden of infection would be to develop a GAS vaccine.

The Dorfmueller group is investigating recombinantly designed *E. coli*–OMVs that include polyrhamnose (pRha) from Group A carbohydrate (GAC) as a potential vaccine candidate against GAS. GAC is expressed by almost all GAS serotypes, and a pilot study using mouse models has demonstrated GAC immunogenicity. Castro presented unpublished work showing that OMVs containing the ubiquitous pRha surface glycan can generate a specific immune response by producing long-lasting OMV-pRha-specific IgG antibodies that correlate with the phagocytic killing of a hypervirulent GAS strain. Increased expression of IgG3 anti-OMV-pRha antibody confirms previous mouse model data on the strong affinity of GAC polysaccharide epitopes. Previous work demonstrated robust affinity of the mouse IgG3 constant region on exposure to repetitive polysaccharide antigens.[121] Furthermore, high surface binding of OMV-pRha antibodies to clinical GAS strains indicates recognition of antigenic epitopes during GAS-dependent infectious disease. This preclinical study supports the conclusion that the GAC polysaccharide leads to stimulation of humoral-mediated antibodies and highlights pRha as a potential vaccine for GAS.

Immune responses to a subunit Klebsiella pneumoniae vaccine

Joseph P. Hoffmann a postdoctoral fellow in the lab of Jay Kolls and Janet McCombs at Tulane University presented data on a subunit vaccine against *K. pneumoniae* developed in the lab. *K. pneumoniae* is notorious for its hypervirulence and drug-resistance mechanisms.[122] As antibiotics become increasingly ineffective, it is clear that other strategies, such as vaccines, will be necessary. Hoffmann described that an ideal vaccine-driven immune response would consist of tissue-resident T cells at mucosal sites that are maintained at sites of pathogen exposure.[123] Hoffmann showed preclinical data on a subunit vaccine composed of the outer membrane protein X (OMPX) of *K. pneumoniae* and LTA1 from *E. coli,* an adjuvant known to generate mucosal responses and drive

IL-17 responses developed and characterized by Elizabeth Norton at Tulane School of Medicine.[124] These IL-17 responses are essential for immunity against *K. pneumoniae.* In mice, the vaccine induced IL-17A$^+$ tissue-resident CD4$^+$ T cells in the lung and reduced bacterial burden in both the lung and spleen. Hoffmann showed that protection was dependent on T_H17 CD4$^+$ cells, as inhibiting pathways necessary for CD4$^+$ cells to differentiate into T_H17 cells abrogated protection. These cells were also able to home to lung and protect against infection in nonimmunized mice. Hoffmann also showed that vaccine efficacy is dependent on IL-17R signaling, specifically in lung fibroblasts. Knocking out *Il17r* in fibroblasts, but not in other cell types, abrogated protection and reduced the number of lung-resident T cells. Finally, since OMPX is highly conserved, the vaccine has the potential to protect across Enterobacteriaceae species. In *in vitro* assays, the vaccine induced an immune response against an array of Enterobacteriaceae species, while it demonstrated protection against *S. marcescens in vivo.*[125]

Accelerating vaccine research with human challenge studies and novel models

Human challenge studies, in which volunteers are inoculated with a pathogen and monitored for a given amount of time, can provide important insights on the immune factors that determine the outcomes of infection. For many respiratory viruses, including SARS-CoV-2, the majority of individuals experience mild-to-moderate disease or even asymptomatic infection. However, patients who fall into certain risk groups, for example, elderly individuals, can have life-threatening disease. It can be difficult to extrapolate these factors for respiratory viruses via animal models or by observational studies of natural infection in humans, which are limited by uncontrolled confounders, lack of analysis of early infection, difficulties in diagnosis and sampling, and heterogeneity among participants. Human infection challenge studies can address many of these limitations since the study defines the viral strain and dose and includes preinfection and early infection assessments. Human challenge studies have been used to study influenza, RSV, and viruses that cause the common cold to infer correlates and mechanisms of protection and accelerate vaccine research and development.[126–128] Human challenge studies can also aid in vaccine development and have typically been conducted during the early stages of development to determine whether vaccine candidates should move on to larger, phase 3 trials. Speakers discussed ongoing efforts to conduct human challenge studies for SARS-CoV-2, *S. pneumoniae,* and GAS.

The UK COVID Challenge Study

Christopher Chiu from Imperial College London discussed the design of an ongoing SARS-CoV-2 human challenge study being conducted in the UK. During the early days of the COVID-19 pandemic, before effective vaccines and therapies were available, human challenge studies were being planned as a way to accelerate vaccine research. In 2021, the WHO published two reports on assessing the ethical acceptability of COVID-19 challenge studies and established a framework for such studies.[129,130] Such studies must obviously have a clear scientific rationale and mitigate the potential risks to participants. Chiu argued that, even with the availability of highly effective vaccines human challenge studies can still provide valuable insights into SARS-CoV-2 infection. In the short term, challenge studies can fill important gaps in our understanding of infection, including factors associated with viral shedding, the incubation period, and impacts of asymptomatic infection on transmission. Longer term, challenge studies can be instrumental for accelerating development of current vaccine candidates. With the availability of effective vaccines, placebo groups in phase 3 studies will soon no longer be an ethically viable option. Head-to-head human challenge vaccine studies can rapidly provide data on the efficacy of new vaccines, new formulations of existing vaccines, or efficacy against viral variants. To mitigate the inherent risks involved, the UK COVID Challenge Study is being conducted in a low-risk population, namely those 18–30 years of age, in whom severe outcomes are rare and symptoms typically resolve within months.[131] Chiu also stressed the importance of extensive public and participant involvement and engagement. Recent surveys and focus groups in the UK show strong support for a human challenge study.

Rationale for pneumococcal challenge studies in Malawi

Stephen B. Gordon from the Liverpool School of Tropical Medicine discussed the rationale for human challenge studies for pneumococcal disease and carriage in Malawi to understand vaccine efficacy. While introduction of the PCV13 vaccine in Malawi has been highly successful and effective in reducing disease incidence and mortality, pneumococcal disease remains a problem for much of the population. Both vaccine and nonvaccine-serotype carriage are high among vaccinated children.[132] People living with HIV, which represent approximately 10% of the population, also show high rates of carriage.[133] Gordon described experimental human pneumococcal carriage studies conducted in Liverpool. Approximately 1800 volunteers took part in 30 independent studies that evaluated three different vaccines and nine pneumococcal strains. In brief, volunteers were inoculated with *S. pneumoniae* through the nose, and nasal samples were collected at various time points to assess whether an individual is a carrier, to quantify carriage density and duration, and to identify immunologic CoPs. The studies evaluated several questions, including the effect of coinfection of *S. pneumoniae* and viral infection and transmission from hand to nose, and developed several different mucosal sampling strategies.

Results from the Liverpool challenge studies demonstrated that volunteers who developed carriage to a given pneumococcal strain were protected from recolonization after reinoculation with the same strain,[134] but not from recolonization from a different strain.[135] If, as in the Liverpool studies, carriage is immunogenic and protective against recolonization with the same serotype, this begs the question of why carriage does not seem to be protecting against recolonization in Malawi.

Gordon stressed that human challenge studies in Malawi can start to answer these questions. With a high carriage rate, particularly among high-risk groups, there is a clear clinical need. Such studies can also leverage over a decade of experience and expertise from the Liverpool studies. In addition, there are several researchers onsite in Malawi with expertise in human challenge studies. In 2017, the Wellcome Trust published a framework for controlled human infection model studies in Malawi,[136] and in 2020 they published a report on the ethical acceptability of such studies.[137] Ultimately, the report concluded that the risks of human challenge studies were largely similar in Malawi and Liverpool, and that such studies could be acceptable in Malawi given that considerations are made with regard to informed consent, inclusion criteria, medical care or support, compensation, regulation, and robust community engagement.[137] The group is moving forward with conducting human challenge studies in Malawi. A feasibility study was recently completed,[138] and techniques to study saliva, nasal fluid, nasal microbiopsies, and nasal biopsies have been transferred to Malawi. The studies will address which vaccines can prevent pneumococcal carriage, how effective they are in vulnerable populations, and the mechanisms of respiratory tract defense needed for an effective mucosal vaccine.

CHIVAS: a model for human challenge studies in Group A Streptococcus

Andrew C. Steer from Murdoch Children's Research Institute presented work on a human infection mode of GAS pharyngitis. As mentioned above, Group A *Streptococcus* is a ubiquitous human pathogen; infection can cause mild, acute symptoms, such as pharyngitis (sore throat), or lead to chronic disease resulting in chronic kidney disease, heart failure, or stroke. Because it only infects humans, GAS is difficult to study in animal models. There is currently no approved vaccine, and only one vaccine candidate has made it as far as phase 2 clinical trials. Steer hopes that human infection studies can be used to bridge phase 1 studies with later clinical trials to accelerate vaccine research.

Steer described the model for such studies, the Controlled Human Infection for Vaccines Against *S. pyogenes* (CHIVAS) study.[139] CHIVAS is a safe and reliable Strep A pharyngitis human infection study in healthy adults that will be used as a platform for learning about bacterial biology and testing vaccines. The model incorporates recommendations from the WHO's preferred product characteristics and vaccine development technology roadmap for GAS vaccines.[118] When choosing the strain to use in CHIVAS, the group considered molecular epidemiology, *in vitro* assays, and the presence of vaccine antigens. The chosen strain, M75, was picked based on its ability to cause pharyngitis but not invasive streptococcal disease.[140] A dose-ranging study

was conducted to define the bacterial dose needed to cause pharyngitis in at least 60% of volunteers and to study systemic and mucosal immune responses. Volunteers were inoculated, received antibiotics, and followed up for 6 months. Of the 20 volunteers inoculated, all demonstrated clinical and microbiological symptoms, with no relapse, carriage, or serious bacteria-related complications.[141] Several groups are now analyzing samples for antibodies, T cells, B cells, mucosal immunity, and cytokines. Future studies will also incorporate vaccines into the CHIVAS model to provide further understanding of the biology of GAS pharyngitis, including CoPs.

Novel 3D infection models

Thomas Rudel from the University of Wurzburg described efforts to develop 3D infection models to use as validation tools for vaccine research. Common infection models for human pathogens include tumor cells, primary cells in 2D culture, and animal models. However, natural infections occur in complex sites composed of several cell types. Rudel's group is part of a large consortium working to develop more natural infection models of sites like the skin, intestine, trachea, and blood–brain barrier. During his talk, Rudel focused on efforts to develop an epithelial model of *N. gonorrhoeae* infection that incorporates interactions with mucus, ciliated cells, and immune cells. Rudel's group has developed several organoid-based models that recapitulate the epithelial properties of fallopian tubes, including ciliated cells with tight junctions. In particular, an air–liquid interface culture system from organoids demonstrates high infectability at the apical side, high cellular differentiation, and long-term infection. Rudel also described a transcytosis model that uses small intestinal submucosa (SIS) as a connective tissue support for primary cells to evaluate the bacteria's ability to travel through the epithelium. Finally, they are incorporating neutrophils into this SIS system to model the transmigration of neutrophils through the epithelium to interact with the bacteria.[142] Together, Rudel's work has established models that incorporate epithelial cells, fibroblasts, a biological scaffold, and immune cells that enable long-term analyses of infection.

Competing interests

The authors declare no competing interests.

References

1. Rappuoli, R. & P.R. Dormitzer. 2012. Influenza: options to improve pandemic preparation. *Science* **336:** 1531–1533.
2. Rappuoli, R. & A. Aderem. 2011. A 2020 vision for vaccines against HIV, tuberculosis and malaria. *Nature* **473:** 463–469.
3. Dormitzer, P.R., P. Suphaphiphat, D.G. Gibson, *et al.* 2013. Synthetic generation of influenza vaccine viruses for rapid response to pandemics. *Sci. Transl. Med.* **5:** 185ra68.
4. Hekele, A., S. Bertholet, J. Archer, *et al.* 2013. Rapidly produced SAM(®) vaccine against H7N9 influenza is immunogenic in mice. *Emerg. Microbes Infect.* **2:** e52.
5. Paden, C.R., Y. Tao, K. Queen, *et al.* 2020. Rapid, sensitive, full-genome sequencing of severe acute respiratory syndrome coronavirus 2. *Emerg. Infect. Dis.* **26:** 2401–2405.
6. Khoury, D.S., D. Cromer, A. Reynaldi, *et al.* 2021. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27:** 1205–1211.
7. Crotty, S. 2021. Hybrid immunity. *Science* **372:** 1392–1393.
8. Koch, T., C. Dahlke, A. Fathi, *et al.* 2020. Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial. *Lancet Infect. Dis.* **20:** 827–838.
9. Jackson, L.A., E.J. Anderson, N.G. Rouphael, *et al.* 2020. An mRNA vaccine against SARS-CoV-2 — preliminary report. *N. Engl. J. Med.* **383:** 1920–1931.
10. Baden, L.R., H.M. El Sahly, B. Essink, *et al.* 2021. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* **384:** 403–416.
11. Wu, K., A. Choi, M. Koch, *et al.* 2021. Variant SARS-CoV-2 mRNA vaccines confer broad neutralization as primary or booster series in mice. bioRxiv 2021.04.13.439482.
12. Wu, K., A. Choi, M. Koch, *et al.* 2021. Preliminary analysis of safety and immunogenicity of a SARS-CoV-2 variant vaccine booster. medRxiv 2021.05.05. 21256716.
13. Folegatti, P.M., K.J. Ewer, P.K. Aley, *et al.* 2020. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* **396:** 467–478.
14. Ramasamy, M.N., A.M. Minassian, K.J. Ewer, *et al.* 2021. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *Lancet* **396:** 1979–1993.
15. Voysey, M., S.A.C. Clemens, S.A. Madhi, *et al.* 2021. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **397:** 99–111.
16. Vasileiou, E., C.R. Simpson, C. Robertson, *et al.* 2021. Effectiveness of first dose of COVID-19 vaccines against hospital admissions in Scotland: national prospective cohort study of 5.4 million people. Available at SSRN: http://doi.org/10.2139/ssrn.3789264

17. Lopez Bernal, J., N. Andrews, C. Gower, *et al.* 2021. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case–control study. *BMJ* **373:** n1088.
18. Oxford University/AstraZeneca vaccine authorised by UK medicines regulator. GOV.UK. Accessed September 17, 2021. https://www.gov.uk/government/news/oxford-universityastrazeneca-vaccine-authorised-by-uk-medicines-regulator.
19. Lopez Bernal, J., N. Andrews, C. Gower, *et al.* 2021. Effectiveness of Covid-19 vaccines against the B.1.617.2 (Delta) variant. *N. Engl. J. Med.* **385:** 585–594.
20. Julia, S., A. Nick, G. Charlotte, *et al.* 2021. Effectiveness of COVID-19 vaccines against hospital admission with the Delta (B.1.617.2) variant: second interim results of a living systematic review and meta-analysis, 1 January to 25 August 2021. Euro Surveill. 2021;26(41):pii=2100920. https://doi.org/10.2807/1560-7917.ES.2021.26.41.2100920
21. Holder, J. 2021. Tracking coronavirus vaccinations around the world. *N. Y. Times*.
22. Tebas, P., S. Yang, J.D. Boyer, *et al.* 2021. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: a preliminary report of an open-label, phase 1 clinical trial. *EClinicalMedicine* **31:** 100689.
23. Mammen, M.P. *et al*. 2021. Safety and immunogenicity of INO-4800 DNA vaccine against SARS-CoV-2: a preliminary report of a randomized, blinded, placebo-controlled, Phase 2 clinical trial in adults at high risk of viral exposure. medRxiv 2021.05.07.21256652.
24. Inovio Pharmaceuticals. 2021. *Phase 2/3 randomized, blinded, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of INO-4800, a prophylactic vaccine against COVID-19 disease, administered intradermally followed by electroporation in healthy seronegative adults at high risk of SARS-CoV-2 exposure*. clinicaltrials.gov.
25. Andrade, V.M., A. Christensen-Quick, J. Agnes, *et al.* 2021. INO-4800 DNA vaccine induces neutralizing antibodies and T cell activity against global SARS-CoV-2 variants. bioRxiv 2021.04.14.439719.
26. McAuley, A.J., M.J. Kuiper, P.A. Durr, *et al.* 2020. Experimental and *in silico* evidence suggests vaccines are unlikely to be affected by D614G mutation in SARS-CoV-2 spike protein. *NPJ Vaccines* **5:** 96.
27. Kanekiyo, M., C.-J. Wei, H.M. Yassine, *et al.* 2013. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature* **499:** 102–106.
28. Yassine, H.M., J.C. Boyington, P.M. McTamney, *et al.* 2015. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat. Med.* **21:** 1065–1070.
29. Powell, A.E., K. Zhang, M. Sanyal, *et al.* 2021. A single immunization with spike-functionalized ferritin vaccines elicits neutralizing antibody responses against SARS-CoV-2 in mice. *ACS Cent. Sci.* **7:** 183–199.
30. Joyce, M.G., W.-H. Chen, R.S. Sankhala, *et al.* 2021. SARS-CoV-2 ferritin nanoparticle vaccines elicit broad SARS coronavirus immunogenicity. bioRxiv 2021.05.09.443331.
31. Joyce, M.G., H.A.D. King, I.E. Naouar, *et al.* 2021. Efficacy of a broadly neutralizing SARS-CoV-2 ferritin nanoparticle vaccine in nonhuman primates. bioRxiv 2021.03.24.436523.
32. U.S. Army Medical Research and Development Command. 2021. *A PHASE 1, randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and immunogenicity of ranging doses of SARS-COV-2-spike-ferritin-nanoparticle (SPFN_1B-06-PL) vaccine with Army Liposomal Formulation QS21 (ALFQ) for prevention of COVID-19 in healthy adults*. clinicaltrials.gov.
33. Shen, X., H. Tang, C. McDanal, *et al.* 2021. SARS-CoV-2 variant B.1.1.7 is susceptible to neutralizing antibodies elicited by ancestral spike vaccines. *Cell Host Microbe* **29:** 529–539.e3.
34. Collier, D.A., A. De Marco, I.A.T.M. Ferreira, *et al.* 2021. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. *Nature* **593:** 136–141.
35. Wibmer, C.K., F. Ayres, T. Hermanus, *et al.* 2021. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nat. Med.* **27:** 622–625.
36. Cele, S., I. Gazy, L. Jackson, *et al.* 2021. Escape of SARS-CoV-2 501Y.V2 from neutralization by convalescent plasma. *Nature* **593:** 142–146.
37. Garcia-Beltran, W.F., E.C. Lam, K. St Denis, *et al.* 2021. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell* **184:** 2372–2383.e9.
38. Kustin, T., N. Harel, U. Finkel, *et al.* 2021. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2 mRNA vaccinated individuals. *Nat. Med*. **27:** 1379–1384.
39. Shinde, V., S. Bhikha, Z. Hoosain, *et al.* 2021. Efficacy of NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* **384:** 1899–1909.
40. Madhi, S.A., V. Baillie, C.L. Cutland, *et al.* 2021. Efficacy of the ChAdOx1 nCoV-19 Covid-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* **384:** 1885–1898.
41. Struyf, F., J. Sadoff & M. Douoguih. 2021. ChAdOx1 nCoV-19 vaccine efficacy against the B.1.351 variant. *N. Engl. J. Med.* **385:** 571.
42. Micochova, P., S. Kemp, M.S. Dhar, *et al.* 2021. SARS-CoV-2 B.1.617.2 delta variant emergence and vaccine breakthrough. *Nature* **599:** 114–119.
43. Kemp, S.A., D.A. Collier, R.P. Datir, *et al.* 2021. SARS-CoV-2 evolution during treatment of chronic infection. *Nature* **592:** 277–282.
44. Choi, B., M.C. Choudhary, J. Regan, *et al.* 2020. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N. Engl. J. Med*. **383:** 2291–2293.
45. Avanzato, V.A., M.J. Matson, S.N. Seifert, *et al.* 2020. Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. *Cell* **183:** 1901–1912.e9.
46. Saunders, K.O., E. Lee, R. Parks, *et al.* 2021. Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses. *Nature* **594:** 553–559.
47. Barnes, C.O., A.P. West, K.E. Huey-Tubman, *et al.* 2020. Structures of human antibodies bound to SARS-CoV-2

spike reveal common epitopes and recurrent features of antibodies. *Cell* **182:** 828–842.e16.
48. Barnes, C.O., C.A. Jette, M.E. Abernathy, *et al.* 2020. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature* **588:** 682–687.
49. Wang, Z., F. Schmidt, Y. Weisblum, *et al.* 2021. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature* **592:** 616–622.
50. Cohen, A.A., P.N.P. Gnanapragasam, Y.E. Lee, *et al.* 2021. Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. *Science* **371:** 735–741.
51. Wilk, A.J., A. Rustagi, N.Q. Zhao, *et al.* 2020. A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat. Med.* **26:** 1070–1076.
52. Wilk, A.J., M.J. Lee, B. Wei, *et al.* 2021. Multi-omic profiling reveals widespread dysregulation of innate immunity and hematopoiesis in COVID-19. *J. Exp. Med.* **218:** e20210582.
53. Hao, Y., S. Hao, E. Andersen-Nissen, *et al.* 2021. Integrated analysis of multimodal single-cell data. *Cell* **184:** 3573–3587.e29.
54. Wimmers, F., M. Donato, A. Kuo, *et al.* 2021. The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination. *Cell* **184:** 3915–3935.e21.
55. 2009. Influenza virus. *Transfus. Med. Hemother.* **36:** 32–39.
56. Krammer, F. & R. Grabherr. 2010. Alternative influenza vaccines made by insect cells. *Trends Mol. Med.* **16:** 313–320.
57. Ekiert, D.C., G. Bhabha, M.-A. Elsliger, *et al.* 2009. Antibody recognition of a highly conserved influenza virus epitope. *Science* **324:** 246–251.
58. Ng, S., R. Nachbagauer, A. Balmaseda, *et al.* 2019. Novel correlates of protection against pandemic H1N1 influenza A virus infection. *Nat. Med.* **25:** 962–967.
59. Hai, R., F. Krammer, G.S. Tan, *et al.* 2012. Influenza viruses expressing chimeric hemagglutinins: globular head and stalk domains derived from different subtypes. *J. Virol.* **86:** 5774–5781.
60. Choi, A., B. Bouzya, K.-D. Cortés Franco, *et al.* 2019. Chimeric hemagglutinin-based influenza virus vaccines induce protective stalk-specific humoral immunity and cellular responses in mice. *ImmunoHorizons* **3:** 133–148.
61. Isakova-Sivak, I., D. Korenkov, T. Smolonogina, *et al.* 2018. Broadly protective anti-hemagglutinin stalk antibodies induced by live attenuated influenza vaccine expressing chimeric hemagglutinin. *Virology* **518:** 313–323.
62. Liu, W.-C., R. Nachbagauer, D. Stadlbauer, *et al.* 2019. Sequential immunization with live-attenuated chimeric hemagglutinin-based vaccines confers heterosubtypic immunity against influenza A viruses in a preclinical ferret model. *Front. Immunol.* **10:** 756.
63. Liu, W.-C., R. Nachbagauer, D. Stadlbauer, *et al.* 2021. Chimeric hemagglutinin-based live-attenuated vaccines confer durable protective immunity against influenza A viruses in a preclinical ferret model. *Vaccines* **9:** 40.
64. Nachbagauer, R., F. Krammer & R.A. Albrecht. 2018. A live-attenuated prime, inactivated boost vaccination strategy with chimeric hemagglutinin-based universal influenza virus vaccines provides protection in ferrets: a confirmatory study. *Vaccines* **6:** E47.
65. Sunwoo, S.-Y., M. Schotsaert, I. Morozov, *et al.* 2018. A universal influenza virus vaccine candidate tested in a pig vaccination-infection model in the presence of maternal antibodies. *Vaccines* **6:** E64.
66. Nachbagauer, R., J. Feser, A. Naficy, *et al.* 2021. A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. *Nat. Med.* **27:** 106–114.
67. Morris, S.E., V.E. Pitzer, C. Viboud, *et al.* 2015. Demographic buffering: titrating the effects of birth rate and imperfect immunity on epidemic dynamics. *J. R. Soc. Interface* **12:** 20141245.
68. Saad-Roy, C.M., C.E. Wagner, R.E. Baker, *et al.* 2020. Immune life history, vaccination, and the dynamics of SARS-CoV-2 over the next 5 years. *Science* **370:** 811–818.
69. Saad-Roy, C.M., S.E. Morris, C.J.E. Metcalf, *et al.* 2021. Epidemiological and evolutionary considerations of SARS-CoV-2 vaccine dosing regimes. *Science* **372:** 363–370.
70. Grenfell, B.T., O.G. Pybus, J.R. Gog, *et al.* 2004. Unifying the epidemiological and evolutionary dynamics of pathogens. *Science* **303:** 327–332.
71. Wagner, C.E., C.M. Saad-Roy, S.E. Morris, *et al.* 2021. Vaccine nationalism and the dynamics and control of SARS-CoV-2. *Science* **373:** eabj7364.
72. Corey, L., J.R. Mascola, A.S. Fauci, *et al.* 2020. A strategic approach to COVID-19 vaccine R&D. *Science* **368:** 948–950.
73. Hammer, S.M., M.E. Sobieszczyk, H. Janes, *et al.* 2013. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N. Engl. J. Med.* **369:** 2083–2092.
74. Neidich, S.D., Y. Fong, S.S. Li, *et al.* 2019. Antibody Fc effector functions and IgG3 associate with decreased HIV-1 risk. *J. Clin. Invest.* **129:** 4838–4849.
75. Janes, H.E., K.W. Cohen, N. Frahm, *et al.* 2017. Higher T-cell responses induced by DNA/rAd5 HIV-1 preventive vaccine are associated with lower HIV-1 infection risk in an efficacy trial. *J. Infect. Dis.* **215:** 1376–1385.
76. Fong, Y., X. Shen, V.C. Ashley, *et al.* 2018. Modification of the association between T cell immune responses and human immunodeficiency virus type 1 infection risk by vaccine-induced antibody responses in the HVTN 505 trial. *J. Infect. Dis.* **217:** 1280–1288.
77. Feng, S., D.J. Phillips, T. White, *et al.* 2021. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat. Med.* **27:** 2032–2040.
78. Arunachalam, P.S., A.C. Walls, N. Golden, *et al.* 2021. Adjuvanting a subunit COVID-19 vaccine to induce protective immunity. *Nature* **594:** 253–258.
79. Zwerling, A., M.A. Behr, A. Verma, *et al.* 2011. The BCG World Atlas: a database of global BCG vaccination policies and practices. *PLoS Med.* **8:** e1001012.
80. Roordink, D., A. Williams, B. Fritzell, *et al.* 2021. The TB vaccine development pathway — an innovative approach to

accelerating global TB vaccine development. *Tuberculosis (Edinb.)* **126:** 102040.
81. Schrager, L.K., J. Vekemens, N. Drager, *et al.* 2020. The status of tuberculosis vaccine development. *Lancet Infect. Dis.* **20:** e28–e37.
82. Hatherill, M., R.G. White & T.R. Hawn. 2019. Clinical development of new TB vaccines: recent advances and next steps. *Front. Microbiol.* **10:** 3154.
83. Nemes, E., H. Geldenhuys, V. Rozot, *et al.* 2018. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. *N. Engl. J. Med.* **379:** 138–149.
84. Van Der Meeren, O., M. Hatherill, V. Nduba, *et al.* 2018. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. *N. Engl. J. Med.* **379:** 1621–1634.
85. Tait, D.R., M. Hatherill, O. Van Der Meeren, *et al.* 2019. Final analysis of a trial of M72/AS01E vaccine to prevent tuberculosis. *N. Engl. J. Med.* **381:** 2429–2439.
86. Rozot, V., E. Nemes, H. Geldenhuys, *et al.* 2020. Multidimensional analyses reveal modulation of adaptive and innate immune subsets by tuberculosis vaccines. *Commun. Biol.* **3:** 563.
87. Dijkman, K., C.C. Sombroek, R.A.W. Vervenne, *et al.* 2019. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. *Nat. Med.* **25:** 255–262.
88. Darrah, P.A., J.J. Zeppa, P. Maiello, *et al.* 2020. Prevention of tuberculosis in macaques after intravenous BCG immunization. *Nature* **577:** 95–102.
89. Blom, K., M. Braun, M.A. Ivarsson, *et al.* 2013. Temporal dynamics of the primary human T cell response to yellow fever virus 17D as it matures from an effector- to a memory-type response. *J. Immunol.* **190:** 2150–2158.
90. Marquardt, N., M.A. Ivarsson, K. Blom, *et al.* 2015. The human NK cell response to yellow fever virus 17D is primarily governed by NK cell differentiation independently of NK cell education. *J. Immunol.* **195:** 3262–3272.
91. Sandberg, J.T., S. Ols, M. Löfling, *et al.* 2021. Activation and kinetics of circulating T follicular helper cells, specific plasmablast response, and development of neutralizing antibodies following yellow fever virus vaccination. *J. Immunol.* **207:** 1033–1043.
92. Brennan, K., B.D. O'Leary, D. Mc Laughlin, *et al.* 2018. Type 1 IFN induction by cytosolic nucleic acid is intact in neonatal mononuclear cells, contrasting starkly with neonatal hyporesponsiveness to TLR ligation due to independence from endosome-mediated IRF3 activation. *J. Immunol.* **201:** 1131–1143.
93. O'Neill, J. 2016. Tackling Drug-Resistant Infections Globally: Final Report and Recommendations May 2016. Accessed June 22, 2021. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.
94. Tomczyk, S., R. Lynfield, W. Schaffner, *et al.* 2016. Prevention of antibiotic-nonsusceptible invasive pneumococcal disease with the 13-valent pneumococcal conjugate vaccine. *Clin. Infect. Dis.* **62:** 1119–1125.
95. von Gottberg, A., L. de Gouveia, S. Tempia, *et al.* 2014. Effects of vaccination on invasive pneumococcal disease in South Africa. *N. Engl. J. Med.* **371:** 1889–1899.
96. Lewnard, J.A., N.C. Lo, N. Arinaminpathy, *et al.* 2020. Childhood vaccines and antibiotic use in low- and middle-income countries. *Nature* **581:** 94–99.
97. Kwong, J.C., S. Maaten, R.E.G. Upshur, *et al.* 2009. The effect of universal influenza immunization on antibiotic prescriptions: an ecological study. *Clin. Infect. Dis.* **49:** 750–756.
98. Andrews, J.R., S. Baker, F. Marks, *et al.* 2019. Typhoid conjugate vaccines: a new tool in the fight against antimicrobial resistance. *Lancet Infect. Dis.* **19:** e26–e30.
99. Lu, E.Y., H.-H. Chen, H. Zhao, *et al.* 2021. Health and economic impact of the pneumococcal conjugate vaccine in hindering antimicrobial resistance in China. *Proc. Natl. Acad. Sci. USA* **118:** e2004933118.
100. Gurnani, V., P. Haldar, M.K. Aggarwal, *et al.* 2018. Improving vaccination coverage in India: lessons from Intensified Mission Indradhanush, a cross-sectoral systems strengthening strategy. *BMJ* **363:** k4782.
101. Choi, E.M. 2021. COVID-19 vaccines for low- and middle-income countries. *Trans. R. Soc. Trop. Med. Hyg.* **115:** 447–456.
102. Patel, M., R.I. Glass, B. Jiang, *et al.* 2013. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. *J. Infect. Dis.* **208:** 284–294.
103. Sindhu, K.N.C., N. Cunliffe, M. Peak, *et al.* 2017. Impact of maternal antibodies and infant gut microbiota on the immunogenicity of rotavirus vaccines in African, Indian and European infants: protocol for a prospective cohort study. *BMJ Open* **7:** e016577.
104. Parker, E.P.K., C. Bronowski, K.N.C. Sindhu, *et al.* 2020. Impact of maternal antibodies and microbiota development on the immunogenicity of oral rotavirus vaccine in African, Indian, and European infants: a prospective cohort study. medRxiv 2020.11.02.20224576.
105. Horn, D. 2014. Antigenic variation in African trypanosomes. *Mol. Biochem. Parasitol.* **195:** 123–129.
106. Engstler, M., T. Pfohl, S. Herminghaus, *et al.* 2007. Hydrodynamic flow-mediated protein sorting on the cell surface of trypanosomes. *Cell* **131:** 505–515.
107. Autheman, D., C. Crosnier, S. Clare, *et al.* 2021. An invariant *Trypanosoma vivax* vaccine antigen induces protective immunity. *Nature* **595:** 96–100.
108. Balhuizen, M.D., E.J.A. Veldhuizen & H.P. Haagsman. 2021. Outer membrane vesicle induction and isolation for vaccine development. *Front. Microbiol.* **12:** 629090.
109. Holst, J., P. Oster, R. Arnold, *et al.* 2013. Vaccines against meningococcal serogroup B disease containing outer membrane vesicles (OMV): lessons from past programs and implications for the future. *Hum. Vaccines Immunother.* **9:** 1241–1253.
110. Petousis-Harris, H., J. Paynter, J. Morgan, *et al.* 2017. Effectiveness of a group B outer membrane vesicle meningococcal vaccine against gonorrhoea in New Zealand: a retrospective case–control study. *Lancet* **390:** 1603–1610.
111. Ferrari, G., I. Garaguso, J. Adu-Bobie, *et al.* 2006. Outer membrane vesicles from group B *Neisseria meningitidis* delta gna33 mutant: proteomic and immunological

comparison with detergent-derived outer membrane vesicles. *Proteomics* **6:** 1856–1866.
112. Berlanda Scorza, F., F. Doro, M.J. Rodríguez-Ortega, *et al.* 2008. Proteomics characterization of outer membrane vesicles from the extraintestinal pathogenic *Escherichia coli* DeltatolR IHE3034 mutant. *Mol. Cell. Proteomics* **7:** 473–485.
113. Gerke, C., A.M. Colucci, C. Giannelli, *et al.* 2015. Production of a *Shigella sonnei* vaccine based on generalized modules for membrane antigens (GMMA), 1790GAHB. *PLoS One* **10:** e0134478.
114. Launay, O., D.J.M. Lewis, A. Anemona, *et al.* 2017. Safety profile and immunologic responses of a novel vaccine against *Shigella sonnei* administered intramuscularly, intradermally and intranasally: results from two parallel randomized phase 1 clinical studies in healthy adult volunteers in Europe. *EBioMedicine* **22:** 164–172.
115. Obiero, C.W., A.G.W. Ndiaye, A.S. Sciré, *et al.* 2017. A phase 2a randomized study to evaluate the safety and immunogenicity of the 1790GAHB generalized modules for membrane antigen vaccine against *Shigella sonnei* administered intramuscularly to adults from a shigellosis-endemic country. *Front. Immunol.* **8:** 1884.
116. Micoli, F., S. Rondini, R. Alfini, *et al.* 2018. Comparative immunogenicity and efficacy of equivalent outer membrane vesicle and glycoconjugate vaccines against nontyphoidal *Salmonella*. *Proc. Natl. Acad. Sci. USA* **115:** 10428–10433.
117. Micoli, F., R. Alfini, R. Di Benedetto, *et al.* 2020. GMMA is a versatile platform to design effective multivalent combination vaccines. *Vaccines* **8:** E540.
118. Vekemans, J., F. Gouvea-Reis, J.H. Kim, *et al.* 2019. The path to group A Streptococcus vaccines: World Health Organization research and development technology roadmap and preferred product characteristics. *Clin. Infect. Dis.* **69:** 877–883.
119. Musser, J.M., S.B. Beres, L. Zhu, *et al.* 2020. Reduced *in vitro* susceptibility of *Streptococcus pyogenes* to β-lactam antibiotics associated with mutations in the pbp2x gene is geographically widespread. *J. Clin. Microbiol.* **58:** e01993–19.
120. Sela, S. & A. Barzilai. 1999. Why do we fail with penicillin in the treatment of group A streptococcus infections? *Ann. Med.* **31:** 303–307.
121. Harmer, N.J. & R. Chahwan. 2016. Isotype switching: mouse IgG3 constant region drives increased affinity for polysaccharide antigens. *Virulence* **7:** 623–626.
122. Logan, L.K. & R.A. Weinstein. 2017. The epidemiology of carbapenem-resistant enterobacteriaceae: the impact and evolution of a global menace. *J. Infect. Dis.* **215:** S28–S36.
123. Schenkel, J.M. & D. Masopust. 2014. Tissue-resident memory T cells. *Immunity* **41:** 886–897.
124. Valli, E., A.J. Harriett, M.K. Nowakowska, *et al.* 2019. LTA1 is a safe, intranasal enterotoxin-based adjuvant that improves vaccine protection against influenza in young, old and B cell-depleted (μMT) mice. *Sci. Rep.* **9:** 15128.
125. Iwanaga, N., K. Chen, H. Yang, *et al.* 2021. Vaccine-driven lung TRM cells provide immunity against *Klebsiella* via fibroblast IL-17R signaling. *Sci. Immunol.* **6:** eabf1198.
126. Habibi, M.S., A. Jozwik, S. Makris, *et al.* 2015. Impaired antibody-mediated protection and defective IgA B-cell memory in experimental infection of adults with respiratory syncytial virus. *Am. J. Respir. Crit. Care Med.* **191:** 1040–1049.
127. Paterson, S., S. Kar, S.K. Ung, *et al.* 2021. Innate-like gene expression of lung-resident memory $CD8^+$ T cells during experimental human influenza: a clinical study. *Am. J. Respir. Crit. Care Med*.
128. Habibi, M.S., R.S. Thwaites, M. Chang, *et al.* 2020. Neutrophilic inflammation in the respiratory mucosa predisposes to RSV infection. *Science* **370:** eaba9301.
129. Jamrozik, E., K. Littler, S. Bull, *et al.* 2021. Key criteria for the ethical acceptability of COVID-19 human challenge studies: report of a WHO Working Group. *Vaccine* **39:** 633–640.
130. Levine, M.M., S. Abdullah, Y.M. Arabi, *et al.* 2021. Viewpoint of a WHO Advisory Group Tasked to consider establishing a closely-monitored challenge model of coronavirus disease 2019 (COVID-19) in healthy volunteers. *Clin. Infect. Dis.* **72:** 2035–2041.
131. Kuiper, V.P., F.R. Rosendaal, I.M.C. Kamerling, *et al.* 2021. Assessment of risks associated with severe acute respiratory syndrome coronavirus 2 experimental human infection studies. *Clin. Infect. Dis.* **73:** e1228–e1234.
132. Bar-Zeev, N., T.D. Swarthout, D.B. Everett, *et al.* 2021. Impact and effectiveness of 13-valent pneumococcal conjugate vaccine on population incidence of vaccine and non-vaccine serotype invasive pneumococcal disease in Blantyre, Malawi, 2006–18: prospective observational time-series and case–control studies. *Lancet Glob. Health* **9:** e989–e998.
133. Swarthout, T.D., C. Fronterre, J. Lourenço, *et al.* 2020. High residual carriage of vaccine-serotype *Streptococcus pneumoniae* after introduction of pneumococcal conjugate vaccine in Malawi. *Nat. Commun.* **11:** 2222.
134. Ferreira, D.M., D.R. Neill, M. Bangert, *et al.* 2013. Controlled human infection and rechallenge with *Streptococcus pneumoniae* reveals the protective efficacy of carriage in healthy adults. *Am. J. Respir. Crit. Care Med.* **187:** 855–864.
135. Pennington, S.H., S. Pojar, E. Mitsi, *et al.* 2016. Polysaccharide-specific memory B cells predict protection against experimental human pneumococcal carriage. *Am. J. Respir. Crit. Care Med.* **194:** 1523–1531.
136. Gordon, S.B., J. Rylance, A. Luck, *et al.* 2017. A framework for Controlled Human Infection Model (CHIM) studies in Malawi: report of a Wellcome Trust workshop on CHIM in low income countries held in Blantyre, Malawi. *Wellcome Open Res.* **2:** 70.
137. Kapumba, B.M., K. Jambo, J. Rylance, *et al.* 2020. Stakeholder views on the acceptability of human infection studies in Malawi. *BMC Med. Ethics* **21:** 14.
138. Morton, B., S. Burr, T. Chikaonda, *et al.* 2021. A feasibility study of controlled human infection with *Streptococcus pneumoniae* in Malawi. *EBioMedicine* **72:** 103579.
139. Osowicki, J., K.I. Azzopardi, C. Baker, *et al.* 2019. Controlled human infection for vaccination against

Streptococcus pyogenes (CHIVAS): establishing a group A *Streptococcus pharyngitis* human infection study. *Vaccine* **37:** 3485–3494.

140. Osowicki, J., K.I. Azzopardi, L. McIntyre, *et al.* 2019. A controlled human infection model of group A *Streptococcus pharyngitis*: which strain and why? *mSphere* **4:** e00647–18.
141. Osowicki, J., K.I. Azzopardi, L. Fabri, *et al.* 2021. A controlled human infection model of *Streptococcus pyogenes* pharyngitis (CHIVAS-M75): an observational, dose-finding study. *Lancet Microbe* **2:** e291–e299.
142. Heydarian, M., M. Schweinlin, T. Schwarz, *et al.* 2021. Triple co-culture and perfusion bioreactor for studying the interaction between *Neisseria gonorrhoeae* and neutrophils: a novel 3D tissue model for bacterial infection and immunity. *J. Tissue Eng.* **12.** 2041731420988802.