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VACCINE INSIGHTS

SPOTLIGHT ON
Clinical development: seizing post-pandemic opportunities to enhance the clinical trial ecosystem

Guest Editor
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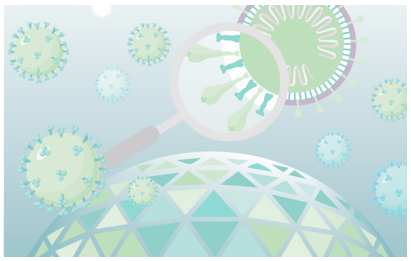
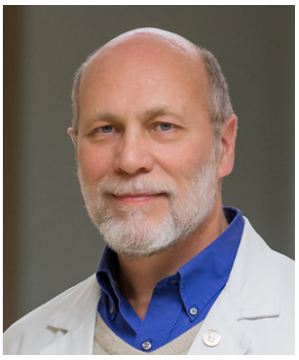


Clinical development: seizing post-pandemic opportunities to enhance the clinical trial ecosystem

INTERVIEW

Lessons from COVID-19: how can vaccine clinical trials adapt to emerging threats?



In this interview, **Charlotte Barker,** Editor, *Vaccine Insights*, speaks to **Daniel Hoft,** PI of the Vaccine and Treatment Evaluation Unit, Saint Louis University (SLU) School of Medicine, about improving pandemic preparedness and the key role of human challenge trials.

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Q How did you get involved in this area of research, and how have your interests evolved?

DH: After college, I worked as a paramedic in Kansas City, and then joined the Peace Corps and served on the island of Borneo as a senior malaria technician. While in Borneo, I saw the huge impact infectious diseases have on the world and that the most cost-effective way of intervening is by developing vaccines.

Throughout my time at medical school, I wanted to go into internal medicine and infectious disease. I won a physician science training award from the NIH and was a research and clinical fellow for 3 years before getting my PhD in Molecular Microbiology and Immunology. My PhD focused on an unusual parasite, *Trypanosoma cruzi*, that causes Chagas disease. This disease is the number one cause of heart disease among Brazilians

and there are about 6–7 million people infected now, mostly in Latin America. After my PhD, Robert (Bob) Belshe, who founded the SLU vaccine center in 1989, asked me to get involved in research on tuberculosis (TB). At the time, there was a US epidemic of TB fueled by the human immunodeficiency virus pandemic. It caught the interest of Bacillus Calmette-Guerin (BCG) vaccine producers, who approached Bob and said, "There might be a market for BCG in the US for the first time in history. Would you test our BCG vaccine and ensure it induces a positive purified protein derivative (PPD) response?" That was what the FDA required for licensure. I noted there are a lot of parallels between Chagas and TB; they are both chronic intracellular infections transmitted through mucosal surfaces and cause disease decades later. I said yes to Bob, and I have now completed around 15–20 clinical trials as the principal investigator for TB vaccines.

We have been part of the Vaccine and Treatment Evaluation Unit (VTEU) network funded by the NIH since 1989 and I have been the team leader for the last decade. The VTEU is a clinical translational, mostly vaccine, trial funding network. There are a lot of microbial threats that could because outbreaks, and we needed to be ready for all of them, so the VTEU network is also a national preparedness network.

In 2009, the network was urgently activated to study the H1N1 pandemic vaccine followed by Zika in 2015, and eventually COVID-19 in 2020. We were diverted from our normal research activities for 2 years to focus on COVID-19 vaccine development, and our previous national preparedness work provided the experience allowing us to start clinical trials within 2 months.

Q What are you and the VTEU working on right now?

DH: We are still working on COVID vaccines. I am the protocol chair for a second-generation COVID vaccine that attempts to induce not only the neutralizing antibody response but also responses from both CD4 and CD8 T cells. We are also learning from COVID in terms of what to do in future pandemics involving other coronaviruses and other pathogens.

Secondly, we are working on universal influenza vaccines. We were all worried that influenza would be the next 100-year major pandemic after 1918, although the coronaviruses beat it, with severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and SARS-CoV-2. We have funding to specifically work on the T cell side of universal vaccine development through a Research Project (R01) grant and earlier awards I received from the NIH.

TB vaccines are another major thing that we have been working on, and we have a vaccine candidate currently in non-human primates.

Q How has the COVID-19 pandemic changed the design of clinical trials and vaccines?

DH: When there is such urgency, things have to be done differently. Everyone in the field of vaccinology has learned so much over the last few years. We always had standard

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ways of testing vaccines. Phases 2 and 3 normally are not even planned until Phase 1 is finished, the regulatory documents are submitted, the data is analyzed, and the results are published. During the pandemic, we did not have that luxury. In 6 months, we had to get as much data as we could for emergency-use approval (EUA).

> "Right now, only a few companies in the world are making challenge agents; they are not available to most academics and companies beyond experimental biology–that needs to change."

Once Moderna and Pfizer COVID-19 vaccines were approved, it was very difficult to develop anything new as they became the gold standards for use in the prevention of COVID-19 infection/disease. We could not enroll subjects into a standard Phase 1 trial with placebo recipients, so we had to figure out new ways to study vaccines. That has mostly been done by studying booster responses with new vaccines in persons previously given COVID-19 approved vaccines. We learned that the mRNA vaccines are great but do not induce long-term neutralizing antibody responses or broadly neutralization effects against mutant variants of SARS-CoV-2 that evolved during the later period of the pandemic.

Q What do we need to do differently in the future?

DH: We cannot become complacent between outbreaks; we need continuous funding focused on pathogens that the scientific consensus suggests as the most likely next pandemic outbreak. That is incredibly difficult; before COVID, coronaviruses were not on my radar, even though we had seen SARS and MERS. We should be funding people to learn about all classes of viruses. Thankfully, because of SARS and MERS, a lot of work had been done on coronaviruses before COVID. This led to mRNA technology coming to the forefront, which is particularly important for rapid vaccine rollout.

People should be looking at different families of viruses, particularly respiratory viruses, and observing what proteins are important for initiating infection. Even if there is no imminent threat, we need to figure out how to take a prototype of a molecule, such as a spike protein from coronaviruses or hemagglutinin from influenza, and optimize the recombinant expression of the protein to expose the epitopes that are most important for induction of broad neutralization of these pathogens.

We should also carry out more challenge studies and generate more challenge agents to allow academics and industry scientists to expand research in two areas. First, experimental biology in humans to learn the targets that can protect people from infectious diseases. We can learn so much from those studies in small numbers of people. The other area to use challenge studies is in testing products in the early clinical pipeline, including both vaccines and drugs. If you have a number of candidates in a major emergency, you need to sift through them and decide what is going to work best. You cannot wait until the end of Phase 3. By testing products in a pandemic early in the clinical pipeline, we can dump things that are

unlikely to make it through Phases 2 and 3. In this way, you can deselect ineffective vaccines and focus on the candidates that are more likely to work.

Right now, only a few companies in the world are making challenge agents; they are not available to most academics and companies beyond experimental biology—that needs to change.

BIOGRAPHY

DANIEL HOFT is the Director of the Division of Infectious Diseases, Allergy and Immunology at Saint Louis University School of Medicine. Since 1992, he has received continuous NIH funding for studies of T cell and mucosal responses to multiple different pathogens, which has helped pioneer the use of immunoinformatic to develop T cell-targeting 'universal' vaccines for intracellular pathogens. In 2014 he became the Principal Investigator of SLU's Vaccine & Treatment Evaluation Unit (VTEU). He has earned national recognition for his contributions to science, including developing improved tuberculosis and influenza vaccines, the development of vaccines for Chagas Disease, and conducting multiple Phase 1–3 COVID-19 vaccine trials. Dr Hoft was elected a Fellow of the St Louis Academy of Science in 2018, and in June 2020, elected to the National Vaccine Advisory Committee, which provides recommendations to DHHS.

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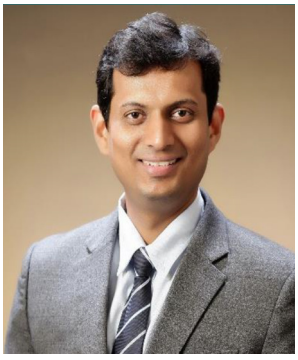
INTERVIEW

Unpacking the vaccine clinical trial ecosystem: implementing adaptive design, leveraging pandemic experience, & supporting resource-limited settings



In this interview, **Charlotte Barker,** Editor, *Vaccine Insights*, speaks to **Sushant Sahastrabuddhe,** Acting Deputy Director General at the International Vaccine Institute (IVI) about his work across the pre- and post-COVID-19 eras of vaccine development, with a focus on resource-limited settings and uniting the global community.

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Q What inspired you to work in vaccine development?

SS: I am a medical doctor by training and started my career in research after graduating from my medical program in India. Working in rural health settings in India, I witnessed many different outbreaks including cholera, typhoid, and dengue. The medical

facilities available were limited and I was always interested in the preventative aspect of global public health, including vaccines. As you are aware, vaccines are one of the most cost-effective tools in global public health.

Q What is the current focus of your work?

SS: I joined IVI 13 years ago, and my role has evolved significantly within the organization. Currently, I wear two different hats. As part of the management team at IVI, I play a role in the organization's strategy and operations, including project and stakeholder management. The second is heading the clinical trial unit, known as the Clinical, Assessment, Regulatory, Evaluation (CARE) unit. We have a team of staff including 40 medical doctors, pharmacists, public health nurses, pharmacists, and accountants who are overseeing clinical trials from Phases 1–3, with vaccines for infections including typhoid, cholera, chikungunya, and COVID-19.

Q How is your work changing post-COVID?

SS: We are venturing out into different sites/countries and expanding our geographical scope. In the initial years, our focus was to conduct clinical trials with the sites that we had in Southeast Asia, South Asia, and Africa. Now, we have extended into Latin America, Central America, Europe, and Oceania. We work in diverse countries having diverse regulatory and operational challenges. A COVID-19 vaccine from a Korean manufacturer, for which we carried out the Phase 3 clinical trial, has just received approval from the UK MHRA, which is exciting for us. We are also performing more adaptive clinical trial designs now.

During COVID-19, many regulatory agencies, including WHO, prioritized study designs and vaccine platforms that were unthinkable before. The worry was, and still is, that regulators around the world will return to the pre-COVID-19 era in terms of approving clinical trials and the approach to risk-taking. In certain clinical trials that we are looking at now, regulators appear to be reverting to traditional ways of doing things. For them, COVID-19 was an exception—it was not business as usual. It will take some time for all regulatory authorities to apply lessons learned from COVID-19 outside a pandemic. Many cannot sustain this pace, because of limitations in qualified staffing. However, there is a willingness from the regulators' perspective to accept unique designs and be more aggressive and risk-taking. We will have to watch and see how this unfolds.

Q How is CARE implementing adaptive clinical trial design?

SS: Pre-COVID-19, most of our trials were done in a traditional manner, transferring the technology to different manufacturers, and performing Phases 1, 2, and 3 clinical trials separately.

The first major trial in which we have applied adaptive clinical trial design is for a chikungunya vaccine that we are working on with Indian manufacturer Bharat Biotech, with funding from the Coalition for Epidemic Preparedness Innovations (CEPI). We planned a Phase 2/3

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trial in three distinct parts. In Phase 2, we will get the safety data for the highest dose group implemented in Phase 1 and repeat that in other countries. Then, we will do age-descending and dose-selection studies within Phase 2. Once we have the results, we will adapt the Phase 3 part of the study within the same protocol, without needing any additional regulatory approval except for data and safety monitoring board review.

> "Almost 60% of the clinical trial centers we are currently working with are new sites in developing countries. These countries provide great opportunities..."

For COVID-19, we are also conducting several studies with non-traditional approaches, including Phase 1/3 trials and Phase 2/3 trials.

Q Is the Asia Pacific region taking a greater role on the global stage for vaccine development?

SS: The profits for vaccines are low compared to those for other medicines and large profits are concentrated on a few vaccines. GSK, Merck, and Pfizer are the top companies in terms of sales and profit by dollar amount. Manufacturers from developing countries do not have those kinds of numbers. In the vaccine business, a major chunk of the profits or sales is taken up by manufacturers based in the US and Europe, rather than those in the Asia-Pacific region or developing countries.

On the other hand, looking at global public health, the Asia-Pacific region is leading the pack. In terms of volume, Indian manufacturers have been producing vaccines for the last 40+ years, and Korean and Chinese manufacturers are coming up very strongly. However, there are significant differences between countries in how this work is funded. In India, the majority of work in the field is private sector-led, with little incentives from the government, whereas in China and Korea, this work is led by commercial entities with support from the Governments.

The Korean government decided to play a major role in the global public health market, partially because of some issues in the procurement of influenza vaccines. The Government started investing steadily in the development of infrastructure, providing incentives for Korean companies with the ambition to have Korean manufacturers be the fifth largest global public health supplier, and they are coming close to that.

Q IVI conducts many of its trials in resource-limited settings, such as Nepal. What are the challenges here?

SS: Almost 60% of the clinical trial centers we are currently working with are new sites in developing countries. These countries provide great opportunities, while at the same time presenting different challenges. The first major challenge in a new country/setting is a lack of understanding of the research environment. For example, Nepal has little experience in Phase 3 clinical trials for vaccines, with the last civilian study of this kind completed in 1986. There were good hospitals, but not much experience with late-stage vaccine

clinical trials. As part of the capacity-building mission of IVI, and with funding from the Bill and Melinda Gates Foundation, we put in place a training program for these sites in Nepal. We shortlisted six sites for training, and the staff underwent 16 months of training to ensure they reached certain capabilities, evaluated by an independent auditor from South Africa. Now, these sites in Nepal are performing multiple Phase 3 clinical trials, including for Sanofi.

The second issue is a lack of a trained workforce, the problem is more prevalent in the developing sites in Africa. As there are more talks and plans for capacity-building in Africa for manufacturing and clinical trials, we need to strengthen these capabilities within Africa using local resources. Training is an ongoing process and resources need to be devoted to making sure that the workforce is not only retained but retrained on the newer developments in the vaccine science. Training is one part of the mandate for IVI because we believe in capacity-building in resource-limited countries.

The third problem is infrastructure and logistics. For example, during monsoons in Nepal, going from one site to another can take days using different modes of transportation.

Q IVI engages in several partnerships with industry—what are the keys to success in these partnerships?

SS: IVI mostly does product development partnerships (PDPs), and we are part of some public–private partnerships (PPPs). For successful PDPs, it is important to have strong governance and a clear steering committee or advisory board to assess progress. We need a clear communication plan for stakeholders, donors, implementing agencies, and the media to be accountable. There needs to be transparent and timely sharing of data as well as technology as part of the PDP. IVI is not for profit, so we do not earn anything from the vaccines that we develop or as part of the partnership with the global public health market.

Q What would you like to take from the COVID-19 experience into potential future pandemics?

SS: In terms of the response as a global community, there were some stark disparities; for example, not having a single dose of the vaccine delivered to many countries in Africa, while some high-income countries had vaccinated much of their population. Global mechanisms, such as COVAX helped, but they were not optimized or impactful enough to have the vaccine delivered to certain countries in time.

Most governments and agencies designated CEPI as the lead in delivering and prioritizing investments for vaccine development, but those investments did not include many manufacturers from developing countries (such as India, Brazil, Korea, or Thailand), which produce more than 60% of the global public health vaccines. To me, this was a big mistake.

This was the first time in modern history that we have had to respond to something as far-reaching as COVID-19. Our response was never going to be perfect, but it could have been made fairer or more equitable.

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BIOGRAPHY

SUSHANT SAHASTRABUDDHE has a total of 20 years work experience in public health and vaccine development projects in multiple countries; 18 years' experience in vaccinology with a certificate in vaccine science and policy and 13 years' experience in vaccine lifecycle management with a focus on vaccine development, policy, and programs. Skills range from clinical medicine to clinical research, epidemiology, biostatistics, surveillance, program management, and fund raising. He is currently leading a team of around 40 scientists (clinical, regulatory, and operational) and administrators to implement vaccine development programs across different parts of the world. The clinical group is leading studies in cholera, typhoid, HPV, chikungunya, COVID-19, HEV, among other pathogens. The grants include development of a novel typhoid conjugate vaccine at IVI (~$29M), chikungunya vaccine ($24M), and COVID vaccine ($45M) and have teams working in various areas of vaccine development (process development, clinical immunology, clinical development, biostatistics, clinical operations, and quality management) working with him.

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VACCINEINSIGHTS

CLINICAL DEVELOPMENT: SEIZING POST-PANDEMIC OPPORTUNITIES TO ENHANCE THE CLINICAL TRIAL ECOSYSTEM

SPOTLIGHT

Developing optimal pediatric COVID-19 vaccines for children younger than 12 years during an evolving pandemic

Flor M Muñoz, Charu Sabharwal, Alejandra Gurtman, Emmanuel B Walter, Grant C Paulsen, Eric AF Simões, Caitlin Hansen, Luke Cunliffe, Stephen Lockhart, Hua Ma, Xia Xu, Kenneth Koury, Kena A Swanson, Nicholas Kitchin, William C Gruber & Annaliesa S Anderson

VIEWPOINT

"With children potentially susceptible to future variants, and calls from the FDA and WHO for an XBB monovalent variant vaccine for the 2023/2024 season, vaccine development will continue to ensure our youngest are protected."

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The COVID-19 pandemic caused unprecedented burden across age groups, economies, societies, and healthcare systems. With the WHO recently declaring the end of the COVID-19 global health emergency [1], it is critical to acknowledge successes and challenges in mitigating the pandemic to ensure preparedness for ongoing COVID-19 outbreaks and emerging variants, and to apply these learnings to future pandemic readiness.

Rapid development of safe and effective COVID-19 vaccines for all age groups was a vital component leading to the end of the COVID-19 emergency. For pediatric populations, vaccine development is inherently more complex, particularly for an infectious disease caused by a dynamic pathogen and when novel vaccine technology has limited prior application in pediatrics. Here we reflect on development of the BNT162b2 mRNA COVID-19 vaccine for immunization of children younger than 12 years.

EARLY PEDIATRIC TRIALS

At the end of 2020, the first vaccine (BNT162b2 30 µg) to prevent COVID-19 was granted emergency use authorization (EUA) based on safety and efficacy results from the pivotal C4591001 trial including participants 16 years and older (Figure 1) [2,3]. The C4591001 trial initiated development in pediatrics with the inclusion of 12–15-year-olds, in whom the vaccine was shown to be safe and effective [4]. These data supported EUA for this age group. Whilst the initial focus was on the adult population and adolescents, the next step in clinical development was progressing in parallel to initiate studies that would gather evidence on safety and efficacy for other populations affected by COVID-19, including children and infants. At the same time, new SARS-CoV-2 variants began to emerge (Figure 1).

BNT162b2 clinical development in <12-year-olds began in March 2021 with initiation of the pivotal Phase 1/2/3 C4591007 trial in pediatrics, which included three age groups (5 to <12 years, 2 to <5 years, 6 months to <2 years) to first determine an appropriate dose level based on the safety and immunogenicity profile for these individual age groups (Figure 1) [5,6]. Two doses of 10 µg given 21 days apart in 5 to <12-year-olds was selected in Phase 1 [5]. However, during dose-finding in the younger age group, approximately 19% of a small group of 2 to <5-year-olds (n=32) who received the 10-µg dose level developed fever after the first and second dose and about one-third of these were severe (>38.9–40.0°C) [6], which was unusual compared with the reactogenicity profile seen in all other age groups [3–6]. In contrast, the 3-µg dose level had a much better tolerability profile combined with good immune response to the SARS-CoV-2 ancestral strain after two BNT162b2 doses [6]. Therefore, for 6-month to <5-year-olds, the 3 µg dose level was chosen for the Phase 2/3 study, which assessed safety, immunogenicity, and efficacy. Noninferior immune responses have been established to infer vaccine efficacy (VE) and were assessed as the primary endpoint in each age group compared with 16–25-year-olds from the C4591001 trial [5,6]. Immunogenicity success criteria were met in 5 to <12-year-olds with a two-dose primary series, the vaccine was safe, and efficacy was demonstrated (observed VE, 90.7% [95% CI, 67.7–98.3]). EUA for this age group was granted during the Delta variant period [5], a variant more antigenically similar to the original SARS-CoV-2 ancestral strain compared with subsequent variants from Omicron sublineages [7].

CHALLENGE: EMERGENCE OF SARS-COV-2 VARIANTS LEADING TO THE NEED FOR THIRD DOSE IN AN ONGOING TRIAL

Beginning in the fall of 2021, the SARS-CoV-2 Omicron variant and its sublineages began to dominate in many regions, demonstrating substantial immune escape from neutralizing antibodies induced by both infection and vaccination [8,9].

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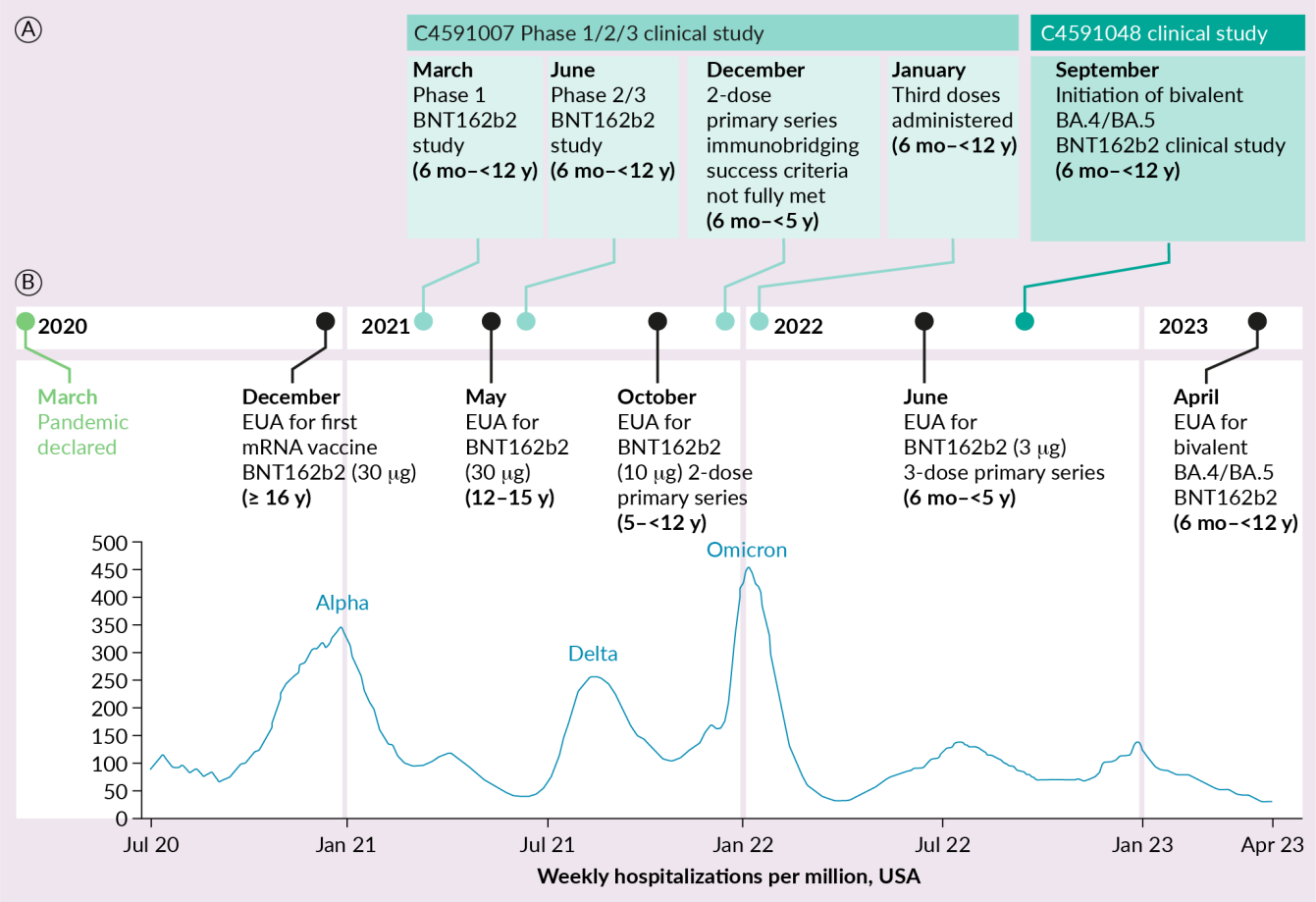
FIGURE 1

(A) Denotes timelines of the period surrounding the pediatric clinical trials for BNT162b2 (C4591007) and bivalent BA.4/BA.5 BNT162b2 (C4591048). (B) Denotes weekly hospitalizations per million people (across all ages) in the USA over approximately the same period, with labels denoting peaks corresponding to COVID-19 variant activity as of May 2023 [17].



In 6-month to <5-year-olds in the C4591007 trial, the initial after dose two immunobridging analysis conducted for each pediatric age group compared with 16–25-year-olds met all immunogenicity success criteria for 6-month to <2-year-olds, but were not fully met for the group of 2 to <5-year-old children [6]. This result, combined with emergence of the Omicron variant and adult data showing an improved Omicron response after a third dose, contributed to the decision in January 2022 to add a third dose for all C4591007 trial participants [6]. Subsequently, immunobridging success criteria were met for the three-dose primary series in 6-month to <5-year-olds; efficacy was also affirmed (observed VE, 73.2% [95% CI 43.8–87.6]) and during a period of Omicron predominance [6]. These results supported an EUA in June 2022.

Given concern regarding waning immunity and emergence of Omicron sublineages with substantial immune escape, the US FDA recommended that all vaccine manufacturers should include an Omicron BA.4/BA.5 component within COVID-19 vaccines [10], prompting evaluation of a bivalent Omicron-adapted vaccine containing coding sequences of both the original and Omicron BA.4/BA.5 spike proteins as a primary series and booster dose in 6-month to <12-year-olds in the C4591048 study (NCT05543616; Figure 1). EUA for primary and booster doses of the bivalent vaccine from 6 months of age was received in April 2023 [11].

The rapid nature of evolving variants with waning immunity resulting in the need for additional doses in all age groups has led to a complex and evolving immunization schedule for vaccine recipients and providers [12]. This may have contributed to poor vaccine uptake in our youngest age groups (in April 2023, 0.8% of 6-month to <5-year-olds and 5.6% of 5–11-year-olds in the USA were up-to-date with their COVID-19 vaccinations), despite >75.3 million COVID-19 cases in those <20-years-old globally, with ~40% of those occurring in children <10-years-old [13,14]. Although children typically have milder symptoms compared with adults [15], risk of infection remains, with children <2-years-old at highest risk of severe illness (an estimated 22.0% of <2-year-olds hospitalized with COVID-19 require ICU admission or mechanical ventilation, or die) [16]. With children potentially susceptible to future variants, and calls from the FDA and WHO for an XBB monovalent variant vaccine for the 2023/2024 season, vaccine development will continue to ensure our youngest are protected.

LOOKING AHEAD

As at the start of the pandemic, early clinical trial initiation remains critical, particularly the ability to include pediatric populations within the pivotal adult study, allowing for operational efficiencies in recruitment of 16–17-year-olds and their inclusion in the original December 2020 EUA. Subsequently, the emergence of variants differing substantially from the vaccine-encoded strain required a third dose in all ages resulting in a delay for our youngest age group (6-months to <5-years-old); however, the knowledge gained from use of the mRNA platform coupled with operational efficiencies will allow for earlier, focused dose ranging for the future. We continue working closely with partners, including governments, practitioners, and public health authorities, to ensure clear messaging on the benefits and risks of vaccinating this age group. These learnings will enable continued protection against COVID-19 and future variants in pediatric populations alongside assuring better future pandemic preparedness.

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Contributions: All named authors take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

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VACCINEINSIGHTS

CLINICAL DEVELOPMENT: SEIZING POST-PANDEMIC OPPORTUNITIES TO ENHANCE THE CLINICAL TRIAL ECOSYSTEM

SPOTLIGHT

EXPERT INSIGHT

Triplex, a viral vectored CMV vaccine for transplant indications: clinical trial updates

Corinna La Rosa & Don J Diamond

Cytomegalovirus (CMV) is a serious complication that increases mortality after solid organ (SOT) or allogeneic hematopoietic cell transplantation (HCT). Immunocompromised patients, including transplant patients, are often unable to mount an effective immune response to contain CMV infection, controlling viral reactivation. There is an unmet need to develop effective therapeutics associated with favorable safety profiles, compared with antiviral therapies for the prevention of CMV end-organ disease and clinically significant CMV viremia after transplant. At City of Hope, our team focused on developing a safe and effective CMV vaccine by using the attenuated vaccinia strain, modified vaccinia Ankara (MVA), genetically modified to express CMV genes. MVA has been investigated as prophylaxis against smallpox and has been approved in the USA and Europe for the prevention and treatment of mpox (monkeypox). This well-established platform for viral vector vaccine development has ample capacity for multiple transgene inserts. Furthermore, it showed an excellent record of tolerability and immunogenicity in immune-suppressed patients and transplant recipients. Based on these multifaceted favorable properties and clinical need, we designed Triplex, an MVA vectored vaccine encoding three immunodominant CMV antigens involved in protective immunity: pp65, IE1-exon4, and IE2-exon5. The purpose of the Triplex vaccine is to rapidly increase CMV-specific T cells after transplant and prevent clinically significant CMV viremia, requiring toxic antivirals. This Expert Insight article presents an integrated overview of Triplex vaccine development pathway from early *in vitro* experiments to pre-clinical testing, manufacturing, production of the clinical lots for first-in-human studies, and pilot and efficacy trials in the transplant setting.

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Human cytomegalovirus (CMV) is a double-stranded DNA virus with the largest genome among human herpesviruses: it contains more than 751 translated open reading frames, encoding over 200 proteins [1]. It has successfully co-evolved with its human host for 200 million years and ubiquitously infects over 90% of the adult population worldwide [2,3]. In the immunocompetent healthy individual, primary infection is mild, yet despite a robust host immune response, the battery of CMV immune evasion and subversion mechanisms enable the virus to establish a latent, life-long infection [4]. Thus, after primary infection, CMV persists under the control of cell-mediated immune surveillance [5–8]. Although CMV-specific T cells do not eliminate the latent virus or preclude transmission, they control viral replication and prevent disease, in the immunocompetent health setting.

CMV infection is the most common infectious complication post-transplant, significantly impacting the success rate of the transplantation procedure and the recovery course of both solid organ (SOT) and allogeneic hematopoietic stem cell (HCT) transplant recipients. In the case of SOT, CMV seronegative patients receiving an organ from a CMV seropositive donor are at the highest risk for uncontrolled CMV viremia leading to end-organ disease and graft rejection [9]. CMV frequently reactivates in CMV seropositive recipients post-HCT, causing serious sequelae which increase morbidity and mortality [10]. Antiviral prophylactic strategies to prevent CMV viral infection or reactivation have significantly lowered the post-transplant risks; however, recipients remain at risk for developing delayed onset of CMV disease, after discontinuation of antiviral prophylaxis [11,12] (>6 months post-transplant). Furthermore, antiviral preemptive approaches provide a targeted use of antiviral drugs and have also shown high efficacy in reducing CMV associated complications [13,14]. Nonetheless, the considerable success of both antiviral strategies is mitigated by multiple drawbacks, including toxicity, significant economic burden, and failure in suppressing CMV viremia [13]. The recently developed prophylaxis treatment with letermovir has greatly reduced myelotoxicity for HCT recipients; however, at conclusion of prophylaxis, delayed onset of clinically significant viremia and resistance are frequent [15,16]. The lack of viral antigen exposure during letermovir prophylaxis likely leads to impaired immune reconstitution [12,17]. It has been recently observed that T-cell responses to immunodominant CMV pp65 and IE1 antigens, which have a major role in controlling CMV infection are markedly decreased in HCT patients receiving letermovir prophylaxis, compared with recipients receiving antiviral preemptive therapy [18]. There is an unmet need for an alternative approach than antivirals for controlling CMV reactivation post-transplant [19,20].

CMV is a highly complex target for the design of an effective vaccine. Multiple global pharmaceutical companies, clinical research organizations, clinical trial companies, and academic research centers are dedicated to the launch of an effective CMV vaccine, which has been ranked as a high priority by the National Institute of Medicine of the USA in 1999 [21,22]. The priority was assigned based on the human suffering and economic costs of congenital CMV infection. Due to intrauterine infection of the fetus or infant, CMV is the leading global cause of congenital abnormalities, such as deafness and other neurological diseases. Moreover, CMV infection can significantly compromise transplantation outcomes and can be life-threatening for immunocompromised persons, including individuals living with HIV/AIDS, patients in critical care, or with inflammatory bowel disease [23–25]. Development of a prophylactic vaccine strategy to attain sterilizing immunity against CMV, providing the highest level of protection, and/or a therapeutic vaccine approach to prevent CMV viremia has been challenging. Currently, no licensed CMV vaccine is available [24].

Since in healthy adults, control of CMV infection is primarily associated with cellular immune responses [26]; consequently, several immunotherapeutic approaches have

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focused on exploiting the natural CMV-specific T-cell response, which is key to lifelong control of CMV [3]. Adoptive immunotherapy based on infusion of CMV-specific T cells can promote durable and functional antiviral immunity after transplantation [27–30]. These findings laid the groundwork for a therapeutic vaccination strategy enabling CMV infection control, by inducing and expanding protective levels of CMV-specific T cells that can limit CMV viremia or disease in post-transplant recipients.

Our academic team has intensively worked for almost two decades to develop a CMV vaccine uniquely designed to rapidly increase CMV-specific T cells after transplant, and prevent clinically significant CMV viremia, requiring toxic antivirals. In this review, we outline the integrated process and the developmental pathway in the design, manufacturing, and production of Triplex.

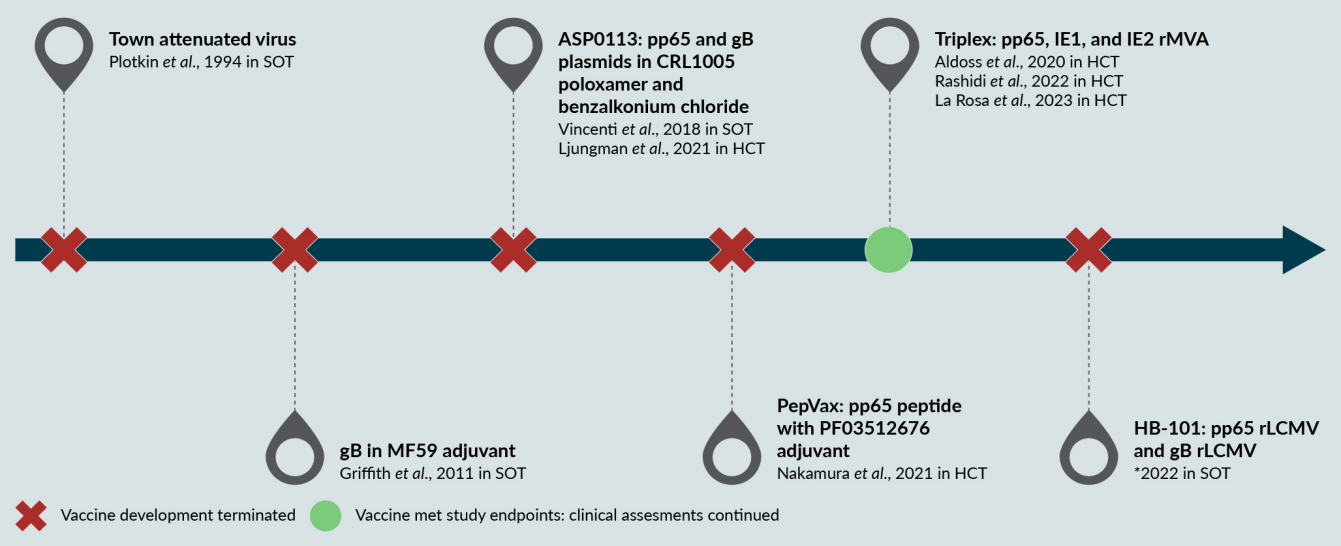
CMV VACCINE PLATFORMS FOR THE TRANSPLANT SETTING

Traditional vaccine development platforms (Figure 1), such as live-attenuated virus, were first designed in the 1970s, as investigational CMV vaccines [21]. The Towne attenuated strain was tested in renal SOT transplant recipients but failed to protect from CMV infection, and the approach was eventually abandoned [31]. The purified CMV surface glycoprotein B (gB) combined with the MF59 oil-in-water adjuvant was also evaluated in SOT recipients. Anti-gB antibody titer significantly increased, though there was no induction of neutralizing antibody, following vaccination [32]. The modest efficacy outcomes did not encourage further clinical testing of this vaccine [33]. A bivalent CMV DNA-based vaccine and a CMV HLA-restricted peptide vaccine both used in combination with adjuvants were the first subunit vaccines designed for the HCT setting. They showed proof of concept that it was possible to safely elicit a CMV-specific cellular immune response by vaccinating HCT recipients [34–36]. Both contained pp65 tegument viral protein, a major target for CMV-specific T cells, with the bivalent plasmid DNA vaccine also expressing the surface antigen, gB. For more than 30 years, reports have emphasized the key role of T cells that target the tegument pp65 protein in protecting immunosuppressed transplant recipients from uncontrolled CMV viremia [37,38]. A direct correlation between recovery of pp65-specific T cells and protection from CMV disease after HCT was found [39]. Adoptive transfer of pp65-specific T cells can effectively treat refractory CMV infection, prevent CMV disease, and control CMV replication and dissemination after HCT [29,40–42]. These milestone data provide a solid rationale for the inclusion of pp65 in CMV vaccines for the transplant setting. Unfortunately, in Phase 2 and 3 trials both CMV DNA- and peptide-based subunit vaccines showed a lack of efficacy in limiting CMV viremia or reducing CMV end-organ disease in HCT [43,44] and SOT [45] vaccine recipients. These disappointing results may be due to the type of vaccine technology used. In general, both DNA and peptide vaccines have limited immunogenicity even if used in combination with adjuvants, and to date, no such vaccines have been licensed for human use [46,47].

Viral vectors can express foreign proteins at high levels in host cells, resulting in strong, long-lasting humoral and cellular immune responses against the target protein [48]. A notable advantage of this platform is that viral vector-based vaccines mimic a natural infection, resulting in the induction of cytokines and co-stimulatory molecules that provide a potent adjuvant effect [49]. Lymphocytic choriomeningitis (LCM) is a rodent-borne viral infectious disease. Replication-deficient viral vaccine delivery platforms, referred to as rLCMV, have proven immunogenic in preclinical animal models [50], do not elicit vector-neutralizing antibody responses, and induce high-frequency CD8 T-cell responses against various antigens. However, boosting of the CD4 T-cell subset is suboptimal, resulting in significantly reduced CD4 T-cell

FIGURE 1

Timeline and development status of CMV vaccine platforms for transplantation.



CMV vaccine platforms for transplantation, assessed in clinical trials are shown. The year of the publication indicates the most recent published clinical trial (fully reported in references) pertaining to each platform, after which development for that vaccine was either terminated (red X symbol) or continued (green dot symbol).
*No publication available as of July, 2023.
gB: CMV surface glycoprotein B; gB rLCMV: Nonreplicating recombinant lymphocytic choriomeningitis virus expressing a truncated isoform of gB; HCT: Hematopoietic stem cell transplantation; IE1: CMV immediate early-exon4 protein; IE2: CMV immediate early-exon5 protein; pp65: CMV phosphoprotein 65 tegument protein; pp65 rLCMV: Recombinant lymphocytic choriomeningitis virus expressing pp65; rMVA: Recombinant modified vaccinia Ankara expressing pp65, IE-exon4 and IE2-exon5 proteins; SOT: Solid organ transplant.

memory subsets [51]. Recently HB-101, a first-in-human vaccine consisting of two rLCMV vectors expressing gB and pp65 was evaluated in CMV-seronegative healthy volunteers [52]. Three injections of the vaccine were well tolerated and induced a gB-neutralizing antibody response, a moderate pp65-specific CD8 T-cell response, but minimal levels of pp65-specific CD4 T cells. A Phase 2 trial in CMV seronegative kidney SOT candidates at high risk for CMV infection did not meet its primary endpoint of reducing viral infection. Hence, further studies with the HB101 vaccine have been suspended. The trial was registered at ClinicalTrials.gov as National Clinical Trial (NCT) 03629080. Though the study clinical outcomes haven't been published so far, the observed lack of efficacy is likely due to the reduced CMV-specific CD4 T-cell response induced by the HB-101 vaccine. During CMV primary infection in healthy adults, CD4 T cells are critical for the resolution of symptomatic disease [53].

Our effort has focused on the use of a recombinant viral vector as a means for vaccination against CMV. Poxvirus vectors have been shown to be highly immunogenic and able to induce robust immune responses. As a viral vector backbone for developing a CMV vaccine to be used in the vulnerable transplant setting population, we choose MVA [54]. Initially developed by Professor Anton Mayr, it is derived from the chorioallantois vaccine Ankara (CVA) strain of the vaccinia virus, passaged over 500 times on primary chicken embryo fibroblasts. As a consequence of these long-term passages, MVA lost approximately 15% of its genome compared to the parental CVA strain [55]. It is highly attenuated, no longer encoding many poxviral immune evasion and virulence factors, and it is propagation-deficient in mammalian cells [56]. Since the packaging defect occurs at a late stage of virion assembly, gene expression remains unimpaired even in non-permissive mammalian cells [56]. Moreover, MVA has a large capacity

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(≥30kb) for foreign gene inserts and can provide high-level gene expression of the insert antigens which result in a potent immunogenic effect [55–57]. MVA has been safely and successfully used as a licensed third-generation vaccine against smallpox, more recently as a vaccine for mpox (monkeypox), and as a recombinant vector for infectious diseases and cancer [54,55,58–61]. Multiple investigations confirmed excellent safety record of MVA even in immunosuppressed individuals [61]. Finally, critical for our choice in the developmental pathway of a CMV vaccine for the transplant setting was that MVA was both highly tolerable and strongly immunogenic when used to vaccinate HCT recipients [62].

TRIPLEX: A CMV VACCINE FOR TRANSPLANT INDICATIONS

To generate a viral vectored CMV vaccine, we obtained the parental wild-type MVA virus (MVA 572.FHE-22.02.1974) by Clinical Trial Agreements from Dr Bernard Moss, Laboratory of Viral Disease (US National Institutes of Health, NIH/National Institute of Allergy and Infectious Diseases, NIAID; Bethesda, MD). At City of Hope (COH) with assistance from the US National Cancer Institute (NCI)-NExT program, we developed Triplex, an attenuated multiple-antigen recombinant MVA with genes encoding three immunodominant CMV proteins (Figure 2): pp65 (UL83), IE1-exon4 (UL123) and IE2-exon5 (UL122) [63]. These viral protein antigens are highly recognized in most CMV seropositive healthy subjects and transplant patients. They can elicit both CMV-specific CD4 and CD8 T-cell responses, which have been described to have key roles in protective immunity, following transplant procedures [64–69]. Triplex was constructed using the MVA viral backbone and two recombinant shuttle vectors, mH5-pp65-pLW51 and mH5-IEfusion-pZWIIA containing all three CMV genes within two transgenes that were inserted into the viral MVA DNA genome using homologous recombination [70]. We fused exon4 from IE1 with adjacent exon5 from the IE2 gene (IE1/e4-IE2-e5) into a single gene (IEfusion) without additional genetic material, to approximate CMV genetic architecture. The fusion protein comprises a more complete representation of the immediate-early antigens than either protein alone. The Triplex vaccine was manufactured, produced, its stability monitored, and quality testing performed at the COH Center for Biomedicine and Genetics (CBG). The CBG is licensed by the State of California's Food and Drug branch as a multi-product biologics manufacturing facility and is subject to US Food and Drug Administration (FDA) inspection.

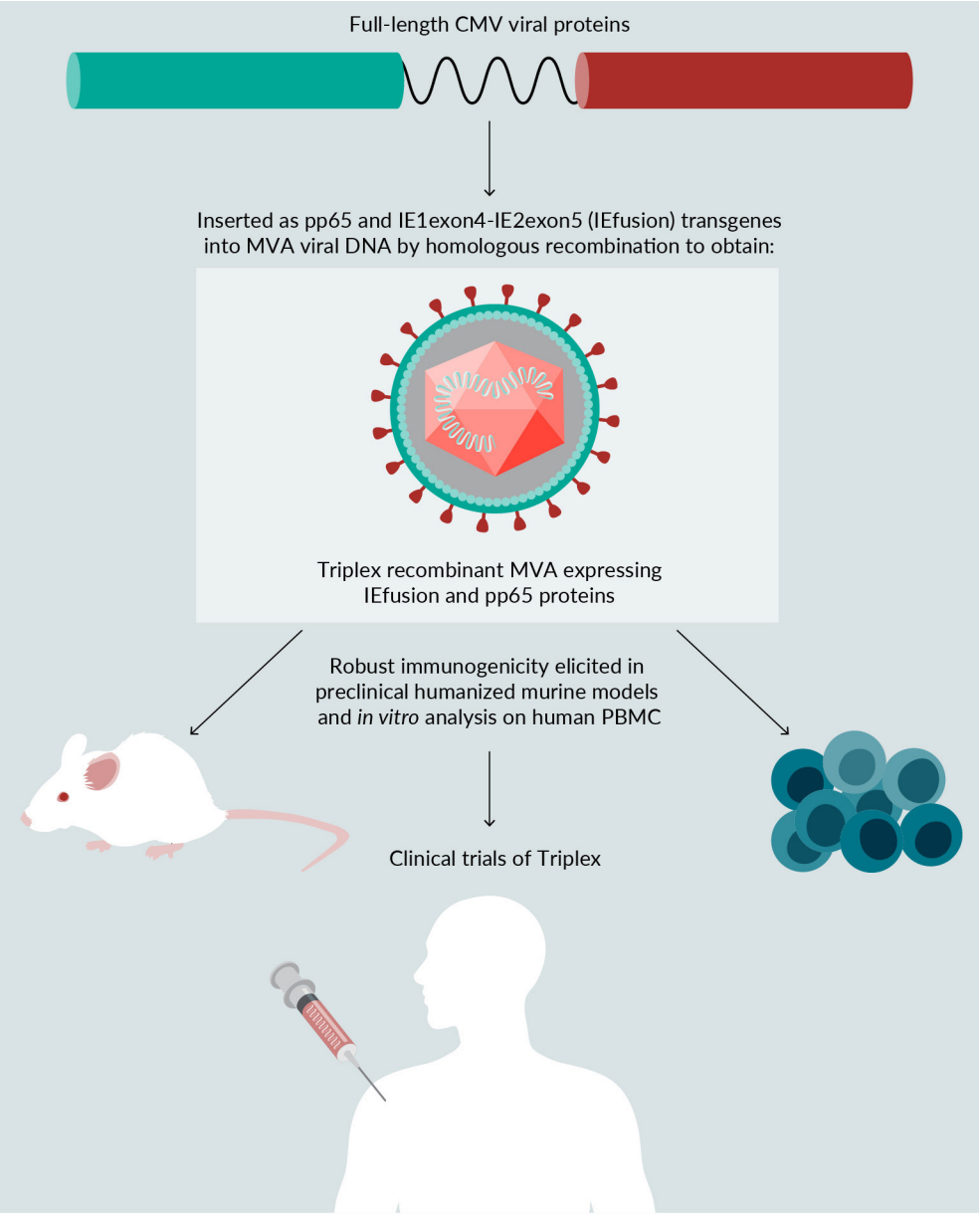
We extensively studied Triplex antigen expression, pre-clinical safety, immunogenicity, and stability using HLA transgenic mice (HLA A2, B7, A1, and A11 humanized mice) [63,71]. The vaccine construct was safe, highly immunogenic, and stable through multiple passages (Figure 3) in all HLA transgenic preclinical murine models used. Furthermore, *in vitro* Triplex amplification of memory T cells present in peripheral blood mononuclear cells (PBMC) derived from CMV seropositive healthy volunteers and HCT recipients showed elevated production of IFN-γ by pp65, IE1- and IE2- specific CD4 and CD8 T cells [63,71].

FIRST-IN-HUMAN CLINICAL TRIAL OF TRIPLEX

Following the successful pre-clinical and *in vitro* testing of the Triplex vaccine, we initiated manufacturing of a clinical-grade lot of Triplex [63]. To support initial clinical development of Triplex, COH filed an investigational new drug application (IND). The FDA permitted the Phase 1 trial of Triplex in healthy volunteers under biologic-based (BB)-IND 15792. The trial was registered as NCT 01941056. This single-center study at COH was designed to assess the safety and immunogenicity of Triplex. Three escalating dose levels (DL) were administered intramuscularly (DL1 = 1 x 10^7; DL2 = 5 x 10^7 DL3 = 5 x 10^8 pfu/dose;) in 8 subjects/DL, with a booster

FIGURE 2

Triplex vaccine development process.



The figure top panel illustrates Triplex vaccine concept and design strategy. In the lower panel, shown is the subsequent developmental pathway, including testing in preclinical murine models and *in vitro* human analyses, which led to the clinical trials.
IEfusion: Immediate early gene regulators IE1-exon4 (UL123) and IE2-exon5 (UL122) which were inserted as single transgene into the recombinant MVA; PBMC: Peripheral blood mononuclear cells.

injection 28 days later, and periodic assessments over the ensuing year for each vaccinee [72]. Triplex vaccine was administered twice in a 28-day period (days 0 and 28) and was shown to have satisfactory tolerability at the highest dose tested. There were no serious adverse events or dose-limiting toxicities. Few injection site reactions were experienced. As MVA is a genetically modified organism, FDA biosafety requirements included monitoring of vector persistence in Triplex vaccinated volunteers. MVA vector persistence in blood, as assessed by real-time PCR showed only minimal residual vector DNA in two vaccinees

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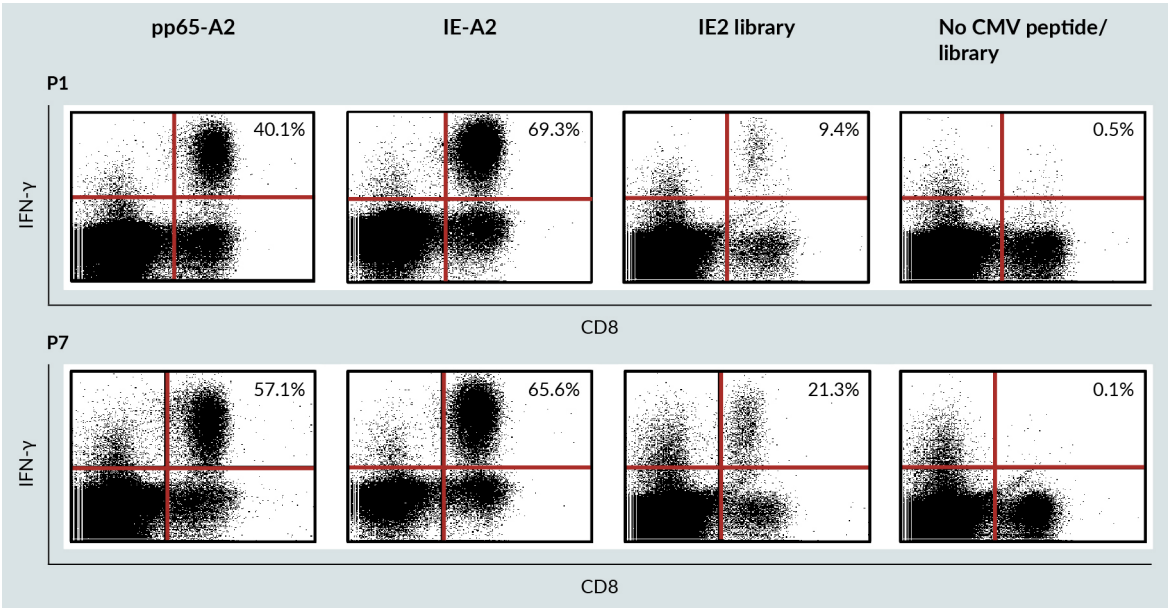
in the DL3 cohort that disappeared within 3 months and did not lead to any adverse event. It was undetectable among all other recipients [72,73]. Immunogenicity was evaluated by measuring T-cell surface levels of 4-1BB (CD137) marker of functional activation and IFN-γ production, combined with memory phenotyping and binding to CMV-specific HLA multimers. At all DL tested (Figure 4), Triplex vaccination induced robust expansion of functional pp65-, IE1- and IE2-specific CD8 and CD4 T cells with predominant long-lived memory effector phenotype, which is associated with viral control during CMV primary infection [74]. Statistical analysis using generalized estimated equations showed post-vaccination levels of pp65 $CD4^+$ and $CD8^+$ T cells were significantly increased and remained elevated 1-year post-vaccination for pp65 [72]. IE1specific T-cell expansions were often noted among participants (Figure 4), although less consistently and generally of smaller magnitude. Triplex vaccine elicited a primary CMV-specific T-cell response in CMV seronegative and enhanced a memory CMV-specific T-cell response in CMV seropositive healthy adults. Elevated and durable CMV-specific T-cell responses were detected in both CMV seropositive and seronegative Triplex vaccinated volunteers, and in subjects who were born before 1973 in the USA, and therefore were presumed to have received mandatory smallpox vaccination [75,76]. Our data are in agreement with several clinical trials of recombinant MVA-based vaccines which have shown that previous vaccination against smallpox had minimal effect on the development of an immune response to an MVA-administered antigen [77,78]. Furthermore, the observed increase of humoral and cellular immune responses to the MVA vector following the Triplex booster [63] did not prevent a contemporaneous increase in the T-cell response to the CMV antigens [75,79].

FIGURE 3

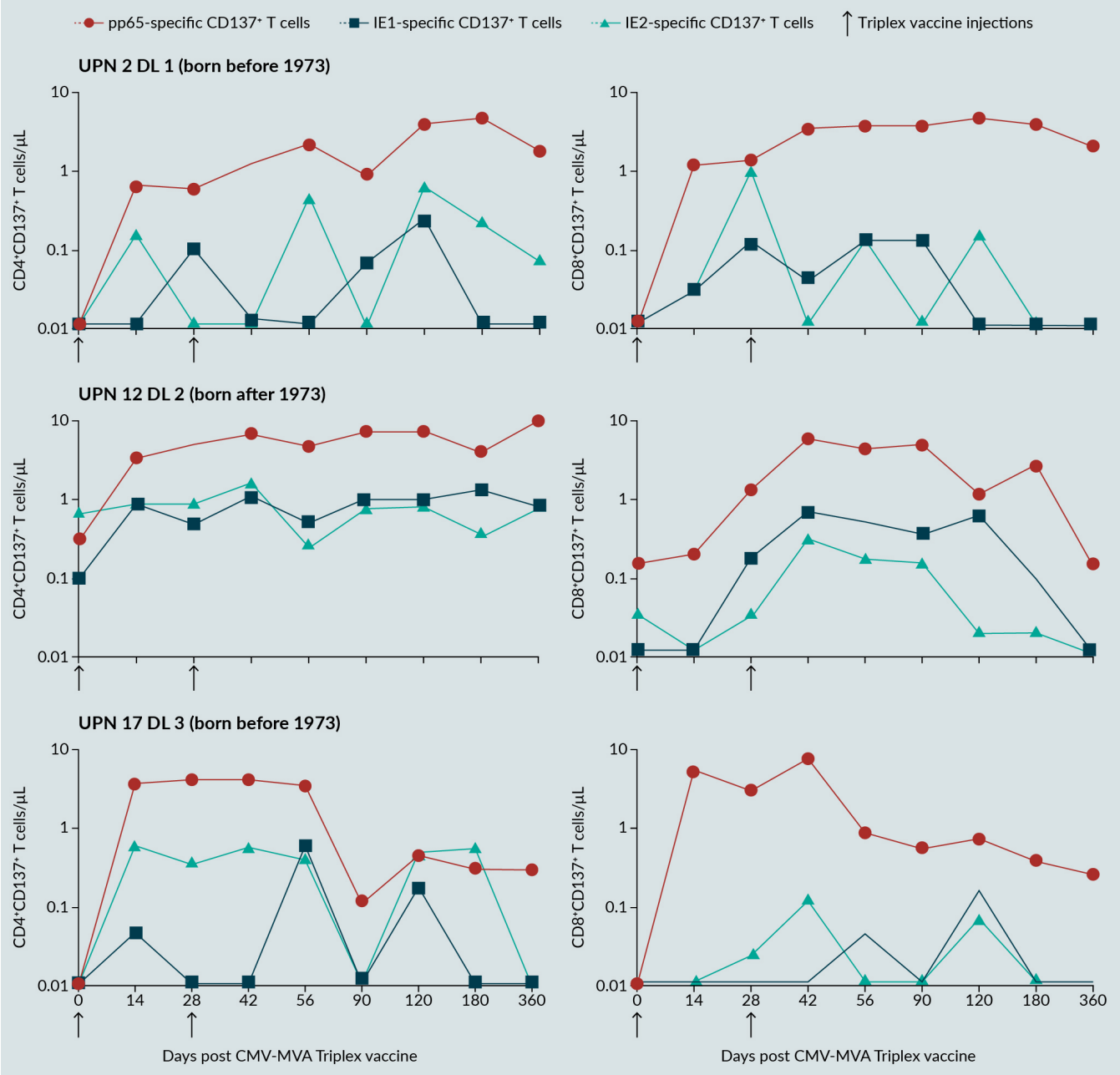
Triplex immunogenicity and stability evaluation in preclinical models.



Immunogenicity of Triplex in viral passage 1 and 7 in humanized transgenic HHD II mice (HLA A2.1), used as preclinical models. Splenocytes from HHD II mice immunized with Triplex from passage 1 (P1, top plots) or passage 7 (P7, lower plots) were *in vitro* stimulated (IVS) as described in Wang *et al.* [63] with either HLA-A*0201 $pp65_{495-503}$ epitope (pp65-A2 in the figure), IE-$1_{316-324}$ epitope (IE1A2) peptides or IE2 peptide library (no HLA-A*0201 IE2 epitope has been described). After IVS, the splenocytes were incubated overnight with pp65A2, IE1A2 peptides, IE2 peptide library or medium as negative control (no CMV peptide/library, in the figure). The immunological activity of the stimulated murine cultures was assessed by measuring levels of CMV-specific IFN-γ production in CD8 T cells by intracellular cytokine staining assays, using multiparameter cytofluorometry as previously detailed in Wang *et al.* [63]. The scatter plots show the percentage of IFN-γ production specific for the CMV antigens indicated (pp65A2, IE1A2 peptides, IE2 peptide library or No CMV peptide/library).

FIGURE 4

CMV-specific CD137⁺ T cells in Triplex vaccinated healthy volunteers.



Immunogenicity of the Triplex vaccine evaluated by longitudinally measuring levels of CD137⁺ T cell surface marker of functional activation in PBMC harvested from CMV seropositive vaccinees and stimulated 24 h with full-length pp65 (obtained from Division of AIDS), IE1 (synthesized in house, blue lines) and IE2 (synthesized in house, green line) overlapping peptide libraries, as described in La Rosa *et al.* [72]. The figure shows the levels of CD4⁺CD137⁺ and CD8⁺CD137⁺ T cells specific for pp65 (red lines); IE1 (blue lines) and IE2 (green lines) in three study participants vaccinated with Triplex, at the dose levels indicated. UPN 2 was born in the United States before 1973 and received mandatory smallpox vaccination.
DL: Dose level; UPN: Unique patient number.

Triplex was the first subunit vaccine to elicit a strong CMV-specific T-cell immune response in CMV seropositive healthy adults (Figure 4). In a CMV seropositive healthy adult, a subunit CMV vaccine likely targets the T central memory compartment, which is under homeostatic control and therefore difficult to alter [80]. For both CMVPepVax

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peptide vaccine and the TransVax™ (Astellas Pharma Inc, Tokyo, Japan) CMV DNA vaccine [81,82], pp65 T-cell responses in CMV seropositive healthy adults were low and detectable only after *in vitro* stimulation. A canarypox vectored vaccine expressing pp65 (ALVAC-pp65 vaccine) induced pp65 T-cell responses only in CMV seronegative subjects [83]. In contrast to ALVAC, early and late transcription are unimpaired in MVA. Hence, there is an extended duration of antigen production in Triplex infected cells, which leads to enhanced immunogenicity [84]. Triplex vaccination did not activate off-target vaccine responses: memory T cells from the ubiquitous and related herpesvirus EBV remained undetectable. These data are in agreement with studies indicating the limited inflammatory response induced by MVA vaccination [85–89]. The favorable safety and immunogenicity outcomes of this study in healthy adults paved the way for a Phase 2 randomized, blinded, and placebo-controlled multicenter trial to evaluate protective function of Triplex vaccine, in CMV seropositive patients undergoing HCT.

A PHASE 2 EFFICACY TRIAL OF TRIPLEX IN HCT RECIPIENTS

We performed a first-in-patient, double-blind Phase 2 trial evaluating Triplex to protect against CMV complications in CMV seropositive recipients with either matched related (MRD) or unrelated (MUD) HCT donor (registered as NCT02506933) [90]. This multi-center study was conducted in three US cancer centers: COH, The Dana-Farber Cancer Institute, and The University of Texas MD Anderson Cancer Center. The target accrual was 102 CMV seropositive recipients randomized 1:1 to either receive Triplex vaccine (5×10^8 pfu/dose) or placebo (n = 51 each arm) on day 28 and 56 post-HCT. Vaccine injections were administered with the intent of eliciting a protective immune response preceding CMV reactivation in the Triplex immunized recipients [91,92]. CMV seropositive HCT recipients are at enhanced risk for CMV reactivation, and more likely to be administered antivirals than CMV seronegatives. The median time to CMV reactivation for HCT recipients is ~40 days, thus our vaccine dosing schedule (days 28 and 56 post-HCT) directly targeted the period of greatest CMV reactivation risk post-HCT [93,94]. CMV reactivation remains the cause of major health complications, profound defects in immune reconstitution, and significant morbidity in the recovery of immune-compromised HCT recipients, diminishing the full curative potential of this successful cancer therapy [91,95]. Primary objectives of this Phase 2 clinical trial were assessing adverse event profile and tolerability of Triplex. Primary endpoint was measurement of CMV events defined as CMV reactivation (viral DNA >1250 IU/ml by qPCR), viremia treated by antivirals, or detection of CMV by tissue histology (end-organ disease). Triplex was highly tolerable, and no safety concerns were related to Triplex injections. The trial met its primary endpoint: a 50% reduction of CMV events in Triplex versus the placebo arm was observed at 100 days post-HCT [90]. Vaccinating HCT recipients earlier than was thought possible for the patient to respond to the vaccine, still yielded rapid, durable, and functional CMV-specific T-cell responses.

The largest vaccine effect on immunity was the recognition of pp65, by both CD4 and CD8 functionally activated T cells. The functionality and antiviral role of CMV-specific T cells have been linked to phenotypic markers describing the level of T-cell differentiation [96,97]. In our memory phenotypic analyses, we found significantly higher levels of CMV-specific T cells displaying the highly functional and long-lasting TEMRA effector memory phenotype in the Triplex arm (Figure 5) compared to the placebo group. CMV-specific TEMRA are subsets of persistently activated effector memory T cells (TEM), which re-express CD45RA after antigenic stimulation [98]. Elevated frequencies of activated CMV-specific TEM and TEMRA cells are associated with a lack of virus detection in the

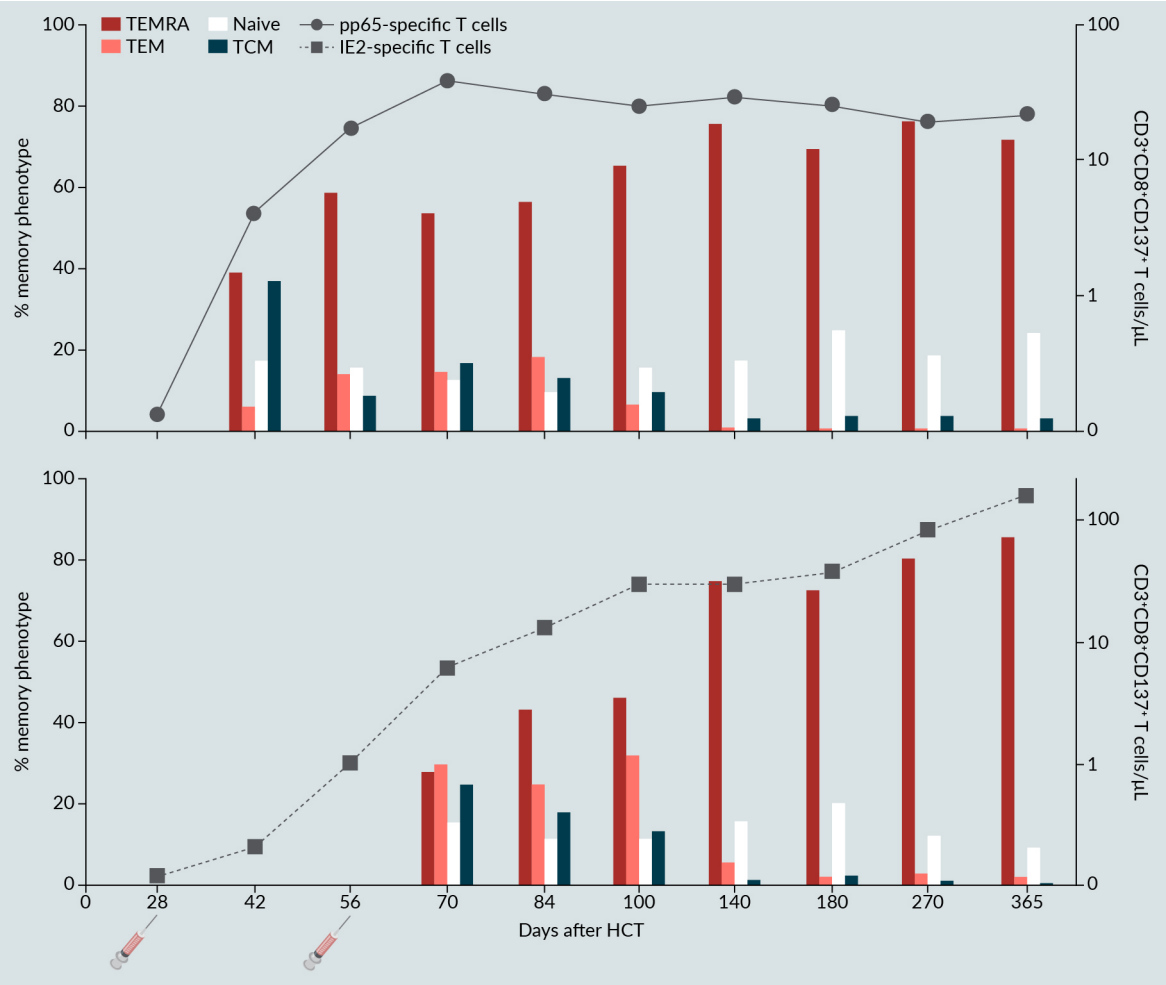
blood of CMV seropositive healthy adults. Hence, in the Triplex vaccinated recipients, the observed increased levels of CMV-specific TEM and TEMRA cells may have played a role in limiting viremia [98]. Triplex is the only CMV vaccine that successfully completed a Phase 2 randomized and blinded trial and caused vigorous and functional CMV-specific T-cell immune reconstitution in the HCT setting.

A PHASE 2 TRIAL IN AUTOLOGOUS HCT RECIPIENTS

In this recently completed study [99] (registered as NCT03383055) at University of Minnesota, we showed that vaccinating CMV seropositive and seronegative lymphoma or myeloma patients after autologous HCT with Triplex safely improved reconstitution of adaptive natural killer NK and

FIGURE 5

CMV-specific T cells and memory phenotype in vaccinated HCT recipient.



Longitudinal levels of pp65- (upper plot) and IE2-specific (lower plot) $CD3^+CD8^+$ $CD137^+$ T cells/µl (right y axes, with logarithmic scale) and their frequency (in percentage, %) of memory phenotype at the indicated days post-HCT, in recipient UPN 41. This CMV seropositive patient received a matched unrelated HCT from a CMV seronegative donor, was born after 1973, was vaccinated with Triplex on days 28 and 56 post-HCT (syringe symbols) and did not reactivate CMV through the one-year study follow up. PBMC at each time point were analyzed by multiparameter flow cytometry, as described in Aldoss *et al.* [90]. When either CMV-specific $CD3^+CD8^+CD137^+$ T cell populations were ≥0.2%, a further analysis for CD28 and CD45RA memory membrane markers was feasible. No memory analysis is reported for IE-1-specific T cells, pp65-specific T cells (upper plot) on day 28, IE2-specific T cells (lower plot) on days 28, 42 and 56 since these $CD3^+CD8^+CD137^+$ T-cell populations were <0.2% in UPN 41. $CD45RA^+$ $CD28^+$ cells were classified as naïve, $CD45RA^-$ $CD28^+$ cells were classified as central memory (TCM), and $CD28^-$ cells were classified as effector T cells. Within the effector T-cell group, two subpopulations were identified: $CD45RA^-$ $CD28^-$ cells (TEM) and $CD45RA^+$ $CD28^-$ effector 'revertant' T cells, re-expressing the RA isoform of the CD45 surface marker (TEMRA).

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CMV-specific T cells. CMV-specific $CD8^+$ T cells and adaptive NK cells have been implicated in graft-versus-tumor-effect and decreased risk of relapse in patients with myeloma [100–102]. The results from this Phase 2 trial are critical for future clinical studies powered to detect the potential impact of Triplex vaccination on relapse rates, in lymphoma or myeloma patients receiving autologous HCT.

A PHASE 1 TRIAL OF TRIPLEX TO VACCINATE HCT DONORS

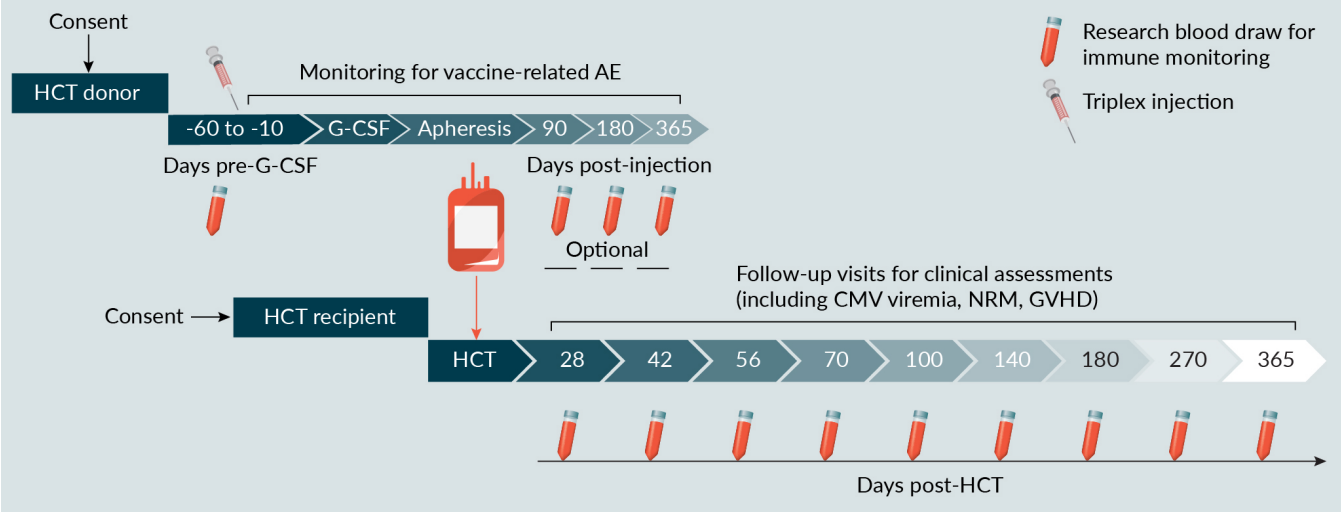
Although the Phase 2 trial in HCT recipients met its primary endpoint, there were early and late CMV reactivation events requiring antivirals in the vaccine arm. Triplex did not prevent and/or control them likely due to the impaired immunologic machinery of the HCT recipient, a consequence of the transplant conditioning regimen. The alternative approach is to upregulate CMV-specific T cells in immunosuppressed recipients of an HCT, by vaccinating their immunocompetent HCT donors with Triplex. The goal of this novel strategy is transferring CMV immunity to the transplant recipient, elicited by vaccination of the immunocompetent HCT donor. Early post-HCT when immune suppressive graft versus host disease (GVHD) treatment [103,104] most frequently occurs, the recipient's ability to mount a vaccine response is compromised and viral reactivation is difficult to control [105]. Safely infusing a graft with enhanced levels of functional and durable CMV-specific T cells can enable the recipient to control CMV reactivation, thereby reducing or eliminating the need for immunosuppressive antivirals.

In the recently published Phase 1 trial (registered as NCT03560752) conducted at COH [106], we assessed feasibility, adverse event profile, and immunological outcomes of vaccinating HCT-matched related donors with Triplex. As illustrated in the graphic schema of the trial (Figure 6), one single injection of Triplex was administered to HCT-matched related donors only. Both CMV seronegative and seropositive HCT-matched related donors were eligible for this study. We investigated whether CMV-specific T-cell immunity elicited by Triplex in the HCT donor could be transferred and expanded in the recipient to prevent or control CMV reactivation. A consequence of clinically significant CMV reactivation is the need for toxic antiviral treatment which can cause significant health complications in CMV seropositive recipients [9,10,107]. This vaccine approach was designed to overcome the early post-HCT immune impairment, when the risk of CMV reactivation is highest [24], and to accelerate immune reconstitution. MRD HCT donors were vaccinated with Triplex once (5×10^8 pfu) before cell harvest. Triplex was well tolerated with limited adverse events in both donors and recipients [72,90,99]. The trial met its primary endpoints: the HCT donor vaccination treatment resulted in augmented frequency of functional and durable CMV-specific T cells, starting early post-HCT and displaying a persistent phenotype of experienced, central memory T cells [106].

The key finding of this novel vaccination strategy is the significantly higher frequency of functionally activated CMV-specific T cells observed early post-HCT, compared to recipients with unvaccinated MRD. Moreover, the donor-derived enhanced T-cell levels were durable and continued to steadily expand during immune reconstitution. Their memory phenotype pattern mainly consisted of antigen-experienced T lymphocytes, which subsequently acquired enhanced effector functions during immune reconstitution [35,36,74,108]. This is a highly favorable immunogenicity outcome, since T-cell-mediated cellular immunity is the most important factor in controlling CMV replication [30,109]. Hence, HCT donor vaccination pre-graft can be a beneficial opportunity for the recipient to receive pools of mature and functional antigen-specific T cells that can accelerate and augment durable immune reconstitution, leading to control of CMV infection post-HCT [110,111]. Though this study was not powered to assess efficacy, CMV reactivation requiring preemptive

FIGURE 6

Trial process of vaccinating HCT donors with Triplex.



The Phase 1 trial schema of Triplex in HCT donors shows the vaccine intervention strategy and the combined clinical and immune monitoring assessments in HCT donor and recipient. One single injection of Triplex was administered to matched related HCT donors only. Both CMV seronegative and seropositive matched related HCT donors were eligible for this study.
AE: Adverse event; G-CSF: Granulocyte colony-stimulating factor; GVHD: Graft-versus-host disease; NRM: Non-relapse mortality.

therapy in recipients with Triplex vaccinated HCT donors was observed to be lower than those in similar cohorts prophylactically treated with the antiviral letermovir [12]. This result suggests that recipient infusions containing abundant and durable donor-derived central memory CMV-specific T cells, leading to enhanced supply of effectors [112,113] could have been the key correlate to reduced CMV reactivation [90,114–116]. The outcome of this Phase 1 trial showed for the first time that the donor vaccination approach is feasible, safe, and successful to increase protective CMV-specific T-cell immunity pre-HCT through donor vaccination, in CMV seropositive recipients.

Feasibility, tolerability, and immunogenicity of vaccinating HCT-matched related donors with Triplex paved the way to the design and planning of a multicenter, randomized, blinded, placebo-controlled Phase 2 trial to confirm the promising Phase 1 findings. A separate clinical study will explore the donor vaccination strategy in the haploidentical (haplo) transplant setting, in which CMV reactivation is among the highest measured (37.5% [12]) after letermovir discontinuation. Haplo HCT donors have been increasingly used when MRD or MUD cannot be identified [117]. These clinical trials in both MRD and haplo HCT settings will be opened to enrollment in the near future and are sponsored by NIH/NCI funding. Their objective is to establish the impact of enhanced specific CMV-specific T-cell immunity on time to reactivation, duration of reactivation, and the possible reduction of cost of care, compared to letermovir prophylaxis. This novel approach has the potential to eliminate or reduce the use of letermovir prophylaxis, overcoming breakthrough resistant viremia [16,118], delays and deficits in T-cell reconstitution [18], and limit morbidity associated with high rates of CMV viremia rebound, requiring toxic antiviral treatment [12,119].

ONGOING CLINICAL TRIALS OF TRIPLEX

Following the favorable outcome of completed trials, Triplex is currently under evaluation in multiple clinical studies in a variety of patient settings.

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1. A Phase 1 clinical study (registered as NCT03354728) at COH to evaluate the optimal dose and the protective effect of Triplex vaccine in pediatric patients receiving an allogeneic HCT or bone marrow transplant (BMT). Preliminary multiparameter cytofluorimetry data analyses [La Rosa *et al.*, unpublished results], in letermovir prophylaxis-treated pediatric participants showed surprisingly robust and functional levels of CMV-specific $CD137^+$ T cells, displaying lasting effector memory phenotype post-Triplex vaccination. The immune profiles suggest that Triplex may prevent uncontrolled CMV viremia. The favorable outcome is possibly linked to the effective activity of the thymus, which is highly functional in children, protecting against serious infections and leading to vigorous vaccine responses [120–122]. These encouraging results laid the groundwork for the design of a multicenter randomized placebo-controlled Phase 2 trial to evaluate the impact of Triplex in promoting protective CMV-immune reconstitution and overcoming letermovir-induced immune impairment. Our long-term goal is to reduce or eliminate the need for immunosuppressive letermovir, as a daily oral medication for these young patients who already have polypharmacy burdens, by enabling the recipient to prevent CMV reactivation by Triplex vaccination.

2. A Phase 2 randomized, placebo-controlled trial (registered as NCT04060277) at COH to evaluate the protective function of Triplex vaccine in adult recipients of haplo HCT. In CMV seropositive haplo recipients, letermovir prophylaxis can initially control CMV viremia but the risk is elevated of developing late-onset clinically significant viremia, CMV disease, and failure to reconstitute CMV-specific immunity [12]. Results of this trial will indicate whether complementing antiviral prophylaxis with Triplex vaccination that harnesses the abundant endogenous immune response to CMV may improve outcomes for these HCT recipients [123]. The unblinding and analysis of this trial is scheduled for the summer of 2023.

3. Pilot/feasibility study (registered as NCT05432635) at COH of CMV-specific CD19-CAR T cells plus Triplex following autologous HCT for patients with intermediate or high-grade B lineage non-Hodgkin lymphoma (B-NHL). To improve the efficacy of chimeric antigen receptor (CAR) T cell therapy, bi-specific viral and tumor antigen CMV-CD19 CAR T cells were manufactured at COH [124–128]. This trial will evaluate the impact of Triplex vaccination on enhancing quality, quantity, and persistence of bispecific CMV-CD19 CAR T cells in infused patients.

4. A double-blind, randomized, placebo-controlled Phase 2 trial (registered as NCT05099965) to evaluate the safety, tolerability, and immunogenicity of Triplex in adults co-infected with HIV and CMV. Evidence suggests that active CMV replication may play a key role in driving progression of HIV-associated opportunistic infections such as those caused by Cryptococcus neoformans and Mycobacterium tuberculosis [129]. Studies have demonstrated that asymptomatic CMV seminal shedding is associated with increased levels of total HIV DNA in both antiretroviral therapy (ART) naïve individuals [130] and in individuals suppressed on long-term ART [131]. CMV shedding is associated with local and systemic immune activation and chronic inflammation with a subsequent increase in the latent HIV reservoir [132]. We hypothesize that the robust cellular immune response induced by Triplex will decrease sub-clinical CMV shedding, systemic inflammation and may also reduce the risk of disease and mortality attributable to opportunistic infections. This study is sponsored by NIH/NIAID and involves investigators from the AIDS Clinical Trials Group.

5. A Phase 2 randomized, multi-center study of Triplex vaccine versus placebo in CMV-negative liver transplants receiving a donor organ from a CMV-positive donor. This trial involves 15 US SOT centers and is supported by NIH/NIAID funding (NCT is pending). There are major limitations in current preventive and therapeutic CMV strategies in high-risk CMV seronegative recipients from seropositive donors. We will test our hypothesis that pre-SOT Triplex vaccination of seronegative liver transplant candidates will lead to improved immune control of CMV and decreases CMV antiviral therapy post-SOT. Patient consent and enrollment will start in the fall of 2023.

CONCLUDING REMARKS

Viral vectors as a means for vaccination against human pathogens are one of the most successful recombinant vaccine technology platforms [48]. They are safer than live attenuated virus vaccines and are more immunogenic than inactivated/killed virus vaccines [133]. They can present the inserted foreign antigens in the natural conformation to the vaccinee immune system, which rarely happens with recombinant protein subunit vaccines [134]. Compared to DNA vaccines, viral vectors express foreign proteins at high levels in host cells, resulting in strong, long-lasting immune responses against the target pathogen protein antigens [49]. However, pre-existing anti-vector immunity may limit efficacy of some viral vectored vaccines, especially those using adenoviruses [135]. Moreover, anti-vector immunity generated after vaccination has been described to impede subsequent booster effect with the same vaccine [136]. Various strategies have been implemented to overcome these hurdles [137]. Triplex is an attenuated poxvirus (MVA) vectored CMV vaccine designed for the transplant setting. MVA has been extensively tested in clinical trials for many years and is accepted as being safe for healthy and immunosuppressed individuals [55,58–62]. Studies in Triplex vaccinated healthy volunteers [72] have shown robust and durable CMV-specific T-cell responses also in subjects who had previously received smallpox vaccination [75]. In further analogy with other recombinant MVA vaccine reports [75], enhanced cellular and humoral responses to the MVA vector after booster injection did not interfere with Triplex-induced CMV cellular immunity [72]. Triplex is currently the only CMV candidate vaccine for transplant indication that met its primary endpoints in Phase 2 trials. Results from completed and ongoing clinical studies confirm that Triplex vaccine is an attractive and versatile immunotherapeutic agent for the transplant setting able to safely elicit, enhance and accelerate protective CMV immunity. Its favorable outcomes and multiplicity of clinical applications are of special interest for infectious disease and transplant physicians, eagerly awaiting effective treatments as alternatives to toxic/immunosuppressive antivirals, for reducing the burden of CMV morbidity in vulnerable transplant recipients.

TRANSLATION INSIGHT

The ongoing clinical development program for Triplex has been the subject of extensive grant funding and collaboration with institutes within the NIH, including NCI and NIAID. Helocyte, Inc. was formed to develop novel immunotherapies for the prevention and control of CMV. Pursuant to a license agreement with COH, Helocyte secured exclusive worldwide rights to develop and commercialize Triplex. Helocyte, Inc. will continue as Sponsor for Triplex, including Phase 3 clinical trials, further development, production, and commercialization.

The process for a candidate vaccine to move from the initial discovery through to licensure generally spans over 15 years, it is complex, multifaceted, and involves diverse and numerous steps. For the development of a CMV vaccine this time lapse has now passed half of a century [24]. Beyond the hurdles, challenges, and complexity of the task, lack of private funders, investor interest, limited federal support, and insufficient sense of

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public health urgency are heavily involved in this extreme delay. We are exiting the COVID pandemic thanks to effective vaccines developed with unprecedented speed, which changed the trajectory of the pandemic. Partnership among researchers worldwide, regulatory flexibility, and concurrent efforts between public and private funding agencies were key to expedite the traditional vaccine paradigm. It's imperative to take stock of these great achievements, which can be applicable with modification to accelerate the production of any valid vaccine candidate, including Triplex.

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INTERVIEW

From HVTN to CoVPN: the importance of vaccine trial networks

Lawrence Corey is a Professor at Fred Hutch and Principal Investigator of the HIV Vaccine Trials Network (HVTN), which conducts studies of HIV vaccines at over 80 clinical trial sites in 16 countries. Early in the COVID-19 pandemic, HVTN took a central role in the COVID-19 Prevention Network (CoVPN), which was responsible for conducting clinical trials of US government-sponsored COVID-19 vaccines and monoclonal antibodies. In this interview, **Charlotte Barker,** Editor, *Vaccine Insights*, speaks with **Lawrence Corey** about coordinating clinical trials during a pandemic and the lessons we can apply to future clinical research.

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Q How has vaccine development changed over the course of your career?

LC: The complexities and costs of all clinical investigations have increased. Now that we have developed vaccines for many of the 'easy' targets, vaccine development has had to become more sophisticated. We have the capability to develop vaccines that protect us, which means preventing us from acquiring the infection in the first place, but it's a harder target than reducing disease severity.

The vaccine field has taken on many new vaccines, and we have had more successes such as the recent respiratory syncytial virus vaccine, but we have also seen significant failures.

You may have assays in the lab that you think are neutralizing assays but too often vitro neutralization doesn't equal *in vivo* neutralization. Efficacy studies for vaccines are large and expensive—how can you de-risk that? It's a fascinating field intellectually.

Q What was your response when your friend and collaborator Dr Anthony Fauci approached you to head the CoVPN?

LC: Well, it was logical. We had a worldwide pandemic, and we needed to develop vaccines as fast as we could. At HVTN, we had built our scientific infrastructure over 20 years to be the largest it had ever been, and our people were well-trained and experienced. It was obvious we should use this infrastructure, so my response was: 'Yes, sir!'.

Q What resources and knowledge from HVTN were you able to apply to the CoVPN?

LC: It was a total pivot. We have a lot of experience designing efficacy studies for human immunodeficiency virus (HIV), but COVID-19 is a very different disease, raising new questions. How many endpoints do you need? What are the endpoints? What is the expected incidence rate? There was a lot of guesswork, but we had some of the world's best statisticians when it came to designing vaccines and analyzing correlates of protection.

We also have an incredible network of clinical trial sites and academic investigators throughout the world. We have built a huge infrastructure in sub-Saharan Africa and a large one in Latin America. Plus, we built community education and community outreach groups to work with communities and involve them.

That investment in infrastructure for HIV trials was critical for the CoVPN because it meant we had a network of trained clinicians who knew how to discriminate between mild and serious disease, follow people sequentially, do pulse oximetry, and draw bloods for correlates of protection.

Q It sounds like you had access to a diverse pool of trial participants—why was that important?

LC: To my knowledge, these were the first US vaccine trials that closely matched the demographic composition of the USA, with more than 20% non-Caucasians. This gave us the data to show that the vaccine worked just as well in Black, Hispanic, and Caucasian people. It was important for people to identify that, 'Yes, it would work in me, and I should go out and get vaccinated'. I hope to see that level of diversity carried forward into future vaccine trials.

Q How did you ensure the trials were completed as quickly as possible?

LC: We started to design the trials even before Operation Warp Speed kicked off. Neither Moderna nor Pfizer/BioNTech were thinking about a 30,000-person trial. They

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were doing two small trials, one in the UK, and one in the US. But we had the advantage of academia—we were not beholden to anyone and could represent the American people, who were ultimately paying for the trials. We wanted to make sure that the trials had incredible veracity and were large enough to underpin public policy.

"The real issue is maintaining that infrastructure, not just for COVID, but for other vaccine-preventable diseases. It would be great to see a series of trials in various respiratory diseases."

The global use of COVID-19 vaccines was largely based on these trials. It was an incredible experiment of essentially putting the same strand of genetic code into different platforms: RNA, viral vector, and proteins. We saw how the platforms made a difference in speed, efficacy, and immune responses. Protein-based vaccines are good, but they took a long time—we had all the results of the mRNA and viral vector trials by the time we got the first proteins into people's arms.

Q What aspects of the COVID experience would you like to see carried forward to improve the vaccine clinical trial ecosystem?

LC: The vaccine clinical trial ecosystem for COVID is terrific, with lots of clinical trial sites and strong community outreach. This was built out of HIV clinical trial infrastructure and has been nurtured. The real issue is maintaining that infrastructure, not just for COVID, but for other vaccine-preventable diseases. It would be great to see a series of trials in various respiratory diseases.

The speed of vaccine development during the COVID-19 pandemic was unprecedented. A large part of that was the virtually unlimited funding we had from the US Government (and ultimately from the US public) and a strong shared purpose. I think COVID-19 is a model of what you can do when you really care.

BIOGRAPHY

LAWRENCE COREY is an internationally renowned expert in virology, immunology and vaccine development, and the former President and Director of Fred Hutch. His research focuses on herpes viruses, HIV, the novel coronavirus and other viral infections, including those associated with cancer. He is Principal Investigator of the HIV Vaccine Trials Network, or HVTN, which conducts studies of HIV vaccines at over 80 clinical trial sites in 16 countries on five continents. Under his leadership, the HVTN has become the model for global, collaborative research. Dr Corey is also the Principal Investigator of the Fred Hutch-based operations center of the COVID-19 Prevention Network, or CoVPN, and co-leads the network's vaccine testing pipeline. Dr Corey's own laboratory group at Fred Hutch studies how immune cells control herpes simplex virus. Their goal is to make a vaccine that will reduce the virus's reactivation. In the early 1980s, he worked with future Nobel laureate Dr Gertrude Elion in the development of acyclovir as the first effective therapy for genital herpes. As director of the AIDS Clinical Trials Group, he led the organization that eventually proved combination antiretroviral treatments could control HIV. The team also demonstrated that these drugs could reduce transmission of HIV from mothers to their infants. His research also showed that HIV-1 replicates in blood early in disease, emphasizing the importance of early therapy.

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