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VACCINE INSIGHTS

SPOTLIGHT ON:

Preclinical & clinical development

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BIOINSIGHTS

Preclinical & clinical development

INTERVIEW

The human vaccines project: into the immunome

The Human Genome Project revolutionized biomedical research. Could mapping the immune system be the next frontier for human health? We caught up with the founder and CEO of the Human Vaccines Project, Wayne Koff, to find out how the initiative aims to decode the immune system and speed up vaccine development.

Charlotte Barker, Editor, Vaccine Insights speaks to **Wayne Koff**, Human Vaccines Project & Harvard TH Chan School of Public Health

Wayne Koff, PhD, is the founding president and CEO of the Human Vaccines Project and adjunct professor of epidemiology at the Harvard T.H. Chan School of Public Health. Prior to joining the Human Vaccines Project, Koff was chief scientific officer and senior vice president of research and development at the International AIDS Vaccine Initiative (IAVI) in New York City for 17 years. Earlier in his career, Koff held leadership roles including vice president of vaccine research and development at United Biomedical Inc. (UBI) and chief of the Vaccine Research and Development Branch, Division of AIDS, at the National Institute of Allergy and Infectious Diseases. An internationally recognized viral immunologist in the field of AIDS vaccine research and development, he has been twice honored by the US Department of Health and Human Services with the Special Act of Service Award for developing innovative strategies for accelerating global efforts in AIDS vaccine development.

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Q What sparked your interest in vaccines?

WK: I was always interested in immunology and viruses – my PhD was focused on flu and my postdoc on dengue viruses. When human immunodeficiency virus (HIV) appeared in the US, I took a position in the AIDS program of the National Institute of Allergy and Infectious Diseases (NIAID), and in 1988 I became chief of the NIAID vaccines branch.

This began a long career in HIV vaccine development – at NIAID, in the biotechnology industry, and at the International AIDS Vaccine Initiative (IAVI), where I led the AIDS vaccine R&D program.

After working on many different vaccine candidates, we ultimately realized that we were not going to make a successful AIDS vaccine until we gained a much better understanding of the human immune system. In the last decade, developments in systems biology, computational biology, artificial intelligence (AI), and machine learning are bringing that understanding within reach.

Q What was the origin of the Human Vaccines Project?

WK: I saw the tremendous impact of the Human Genome Project (HGP) and felt there was a parallel between where immunology is today, and where genomics was in the late 1980s when the HGP was first posited. Could an equivalent effort advance immunology in the same way the HGP has advanced genomics, and lead to new therapies?

A seminal meeting in 2014 brought together 35 immunologists and vaccinologists from industry and academia worldwide to flesh out the idea [1,2], and a series of workshops addressed specific issues like the human immunome and the human antigenome.

Two years later, we officially launched the Human Vaccines Project – a global non-profit consortium of academic laboratories and industry partners dedicated to mapping the complexity of the immune system to develop better vaccines.

Q What approach are you taking?

WK: We run comprehensive immunologic and systems biology analyses on licensed vaccines, aiming to understand the pre-vaccination baseline that is predictive of outcome. As an example, the hepatitis B vaccine has excellent efficacy – after three doses, 85–90% of people are protected. However, there is variation; a small number of people are not protected after three doses and, interestingly, about a quarter of people are protected after a single dose.

These studies are telling us that there is a bell-shaped curve – most of us have a relatively efficient immune system, but there are some individuals whose immune systems are either highly active or suppressed. We are attempting to understand these differences at the molecular level and apply that knowledge to vaccine development.

The ambitious long-term goal of the Human Vaccines Project is the development of AI models of the human immune system. Vaccines require large efficacy trials with tens of thousands of individuals. Generally, there is only a modest amount of immunology done in those trials; for example, looking for a few markers of antibody titer or neutralizing antibody. We're asking whether we can move this work *in silico*. If we can generate the requisite data to enable the machines to create the models, will we be able to predict how well a vaccine will perform?

We envision a time when we have enough information on individuals' immune systems that *in silico* models could run a hundred thousand (virtual) vaccine trials in a day. On

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the development side, it provides an opportunity to make vaccine development faster and cheaper, with a greater probability of success. On the discovery side, it would give a whole generation of scientists a better understanding of the human immune system and a new set of tools to ask questions they haven't been able to before.

We're focusing on vulnerable populations. One of our initiatives is a partnership with colleagues at the Harvard TH Chan School of Public Health looking at immune function in the elderly; another that we are in the process of launching is the Born Strong Initiative, with the Telethon Kids Institute (Perth, Australia) focused on optimizing maternal and newborn immunity.

A decade from now, it might be possible to have everyone's immunome sequenced, just as it will soon be feasible to have everyone's genome sequenced. This is a long-term, ambitious project, but there are many opportunities along the way.

Q What are the next steps?

WK: One part of the project is the huge sequencing effort of the B and T cell receptor repertoires already underway; the second part is a systems biological effort, integrating all the data from studies with licensed vaccines using AI modeling. Beyond just sequencing and understanding the basic shape of an immune receptor, our goal is to predict what that B cell or T cell is going to bind to.

We have set up a human immunology network across the world, and have conducted comprehensive studies on the human immunome. We have identified some of the biomarkers needed to predict baseline immunity and we are now in the process of creating a database that we can hand over to the AI experts of the world. We will then be able to ask questions, like why do only some people get a disease? And why do some people respond better to vaccines?

The next steps depend to some degree on the limitations of the technologies needed. There is exponential advancement, but we do not yet know exactly how far and how fast the field of AI will progress. We do know that the many breakthroughs this past year were in predicting the structure of a protein, based on its sequence. If that is where we are now, when and how can we get to a structural map (rather than a sequence map) of the human immunome?

Q What are your hopes (and fears) for the future?

WK: I think we are at the dawn of the golden age of vaccine development. The new platforms that have now been shown to be effective are going to bear fruit for the development of vaccines in both the infectious and non-communicable disease spaces.

Idealistically, I would hope that within a decade we will have the initial, albeit perhaps primitive, AI model of the human immune system. I believe we can achieve it if we can generate the enthusiasm and resources needed and get the immunologists, system biologists, and AI scientists all working together in a global consortium.

Having been around the block for a long enough time, I know that the political landscape is such that long-term projects are always challenging. I fear that we are not going to take

the lessons of the COVID-19 pandemic to heart; we will fall back into apathy and when the next pandemic threat comes along, we will have missed an opportunity. I hope that we have learned our lesson and that we will act more and talk less in future. We have the opportunity to make the impossible possible, and transform the future of human health.

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VACCINEINSIGHTS

PRECLINICAL & CLINICAL DEVELOPMENT

SPOTLIGHT

EXPERT INSIGHT

Opportunities & challenges of applying AI/ML to integrating systems vaccinology studies

Robert A van den Berg & Vanesa Bol

Vaccines are essential tools in the control of infectious diseases. The development of vaccines has matured from a mostly empirical to a highly sophisticated approach that is science-grounded and based on state-of-the-art knowledge in molecular biology, immunology, and structural biology. Insights in the mechanism of action of vaccines have been driven by clinical research studies that leverage systems vaccinology approaches to unravel the links between the innate immune response and the quality and persistence of adaptive immunity. Over the last decade an increasing number of clinical systems vaccinology studies have been published. In parallel, developments in artificial intelligence and machine learning (AI/ML) have made a massive impact in real-life applications from image classification to speech recognition. AI/ML has also entered the world of vaccinology. In this manuscript, we reflect on how AI/ML could be used to leverage the wealth of clinical systems biology data to drive novel insights in vaccines mechanism of action.

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Vaccines are highly effective tools to prevent the spread of infectious diseases and to minimize their impact on human health, as illustrated by the COVID-19 pandemic. Until recently, vaccines were developed empirically by isolating target pathogens, attenuating their virulence and purifying their proteins or by preparing suspensions of killed viruses or bacteria [1]. With the advent of molecular biology, vaccine development expanded

significantly and, to-date, a large number of vaccine platforms are available, such as virus-like particles, adjuvanted vaccines, synthetic peptide, polysaccharide, polysaccharide conjugate (glycoconjugate), or viral vectored and nucleic acids (DNA and mRNA) used to express the target antigen [2]. The mode of action of these vaccines is still partly unexplored. The immune response induced by these platforms is highly complex and multifactorial. The events occur through space and time as the injection of the vaccine alerts muscle and local immune cells of the presence of a foreign entity. This triggers a cascade of events mediated through signaling molecules and cell-to-cell interactions. Immune cells are recruited to the site of injection and the lymph node. Vaccine is transported to the lymph node either by direct flow or through antigen presenting cells [3]. There, the immune microenvironment drives the activation and maturation of T and B cells that drive the development of the adaptive response. Over a period of several weeks, these T and B cell develop into effector and memory types to address the initial exposure and be ready for future ones. However, key questions in this process remain open. For instance, what mediates vaccine efficacy and adverse reactions, or how to combine the best platform and antigen to solicit the strongest response to a certain pathogen, or which immune micro-environment drives long-lasting protective immune responses [4–6].

Systems vaccinology offers a new approach to vaccine-related questions by analyzing the molecular pathways involved in a vaccine response, which could lead to identifying global correlates of successful vaccination, new methods for measuring early vaccine responses, and ultimately to generating hypotheses for understanding the mechanisms that underlie successful immunogenicity [7]. With omics-type of data, specific molecular signatures can be identified and used as predictors of vaccine mode of action or vaccine efficacy [8–10]. New high-throughput technologies have further enhanced the possibilities for studying an increased number of genes or increased granularity with single cell technologies, generating large volumes of data. Of specific interest for the vaccine field is the characterization of early inflammation induced by vaccination and identification of biomarkers of reactogenicity [11]. However, only few publications cover this field. Of interest, within the BIOVACSAFE consortium, a public private consortium of 19 partners involving experts from academia, non-governmental organizations, and vaccine companies, the authors analyzed data from three vaccine studies and showed that the inflammatory response was rather a function of both the vaccine composition (live vs. inactivated; nonadjuvanted vs. adjuvanted) and the presence of immune (CD4 T cell or B cell) memory in the recipient [12] .

THE BEGINNING OF SYSTEMS VACCINOLOGY

Since the early days of applying systems vaccinology to predicting immunogenicity of yellow fever vaccine [13, 14], the question of whether such approaches could also be applicable beyond individual studies is still to be answered. Indeed, existing signatures and observations rely on small cohorts and have not been validated in large independent studies. Only a few studies attempted to integrate data from different cohorts to answer specific questions like in Nakaya et al. [15], where the authors analyzed the innate and adaptive responses to seasonal influenza vaccination in humans and defined early predictors of the vaccine-induced antibody response in two independent influenza trials with trivalent inactivated influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV). In another report, researchers have integrated innate and adaptive immune responses from different vaccines, against malaria, human immunodeficiency virus (HIV) and tuberculosis (TB), and demonstrated that general principles exist which relate innate and adaptive immune responses across multiple vaccines [16]. In a third example, the authors leveraged

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multiple influenza vaccination cohorts to identify baseline predictive transcriptional signatures of influenza vaccination responses. The multicohort analysis identified genes and modules significantly associated with the magnitude of the antibody response with an inverse correlation between the effect size of signatures in young and older individuals [17]. More recently, in a paper describing the systems vaccinology analysis for BNT162b2 SARS-COVID mRNA vaccine in humans, the authors performed an analysis comparing a set of published vaccine trials with the vaccine of interest and demonstrated that there is a broadly similar response to vaccination between adjuvanted vaccines (H5N1 + AS03), live viral vectors (Ebola and HIV vaccines), and inactivated influenza, which stimulates a recall response, at Day 1 post booster, but not after prime [18]. Finally, researchers from the Human Immunology Project Consortium (HIPC) developed a curated database of immune signatures [19] and explored interrelations between the vaccines in this database [20]. They made a comparative analysis of transcriptional responses from 820 healthy young adults across 13 different vaccines and found that although a common transcriptional signature can be shared among different vaccines, there is significant heterogeneity especially in the kinetics of immune response.

One of the prerequisites for performing such integrative analyses is the availability of data sets of interest in public/open-access repositories allowing researchers to reuse these data for secondary analyses. Common repositories are GEO and ArrayExpress, which accept only array- and sequence-based data [21, 22], flowrepository.org [23] for flow cytometry data, and ImmPort, which is dedicated to subject-level human immunology data beyond only arrays and sequences [24]. To date, more than 500 studies have been made freely available through ImmPort's Shared Data portal (ImmPort Shared Data), which allows research data to be repurposed to discovery of new insights of future vaccines.

Another important factor for re-use of data is the availability of information concerning the analytical methods and algorithms that were used. Together with the metadata, these are critical but rarely linked to data repositories. The repositories tend to be a rather static environment where data is dropped by study/collection. The work by the HIPC researchers shows the effort required to bring these kinds of data together [19], as we will discuss later.

AI/ML FOR SYSTEMS VACCINOLOGY

The maturation of AI/ML

In parallel to the maturation of the systems vaccinology field, the domain of artificial intelligence and machine learning (AI/ML) has come to fruition and has been used in various real-life applications, such as, image classification and voice recognition [25, 26]. The maturation of AI/ML was made possible by technological advances in other areas, notably the massive growth of available data available for learning and access to scalable computer power through the development of GPUs and cloud computing. Following this, the AI/ML applications and research expanded in various directions, including life sciences.

There are several advances with potentially profound impact on vaccines R&D. For example, Alphafold [27] established a new standard for protein structure prediction from sequence. Furthermore, language models were used to predict viral evolutionary escape routes of the SARS-COV-2 Spike protein [28]. Combined with high throughput technologies, such as, deep mutational screening [29, 30], these advances will shape the future of antigen design. Also in the area of B and T cell epitope prediction, AI/ML takes advantage of the expanding data availability [31–34].

Applications of AI/ML in systems vaccinology have also emerged, particularly in single cell RNA sequencing [35]. Here, AI/ML methods capitalize on the high data density per sample at single cell resolution to address different challenges that accompany

this methodology, like identification of clusters, removal of unwanted experimental artefacts, cell annotation, and integration with other data sources or measurements in the multi-omics context [36–39]

While these examples highlight how AI/ML technology is finding its way into vaccinology, we did not yet find examples of the use of AI/ML for the integration of data across clinical systems biology studies.

AI/ML for the integration of clinical systems vaccinology studies

The promiscuous use of AI/ML begs the question how AI/ML can be applied to address the open questions in the study of vaccine mechanism of action capitalizing on the available clinical systems vaccinology studies. In an ideal world, the numerous clinical systems vaccinology studies that have been published, e.g., [12–15, 19, 40–42] and others, would be captured in a unifying model describing early and adaptive immune signatures after vaccination. This would be a treasure trove for academic and corporate scientists alike.

At first glance, it looks like there is a good fit between the attributes of AI/ML models and the availability of clinical systems vaccinology studies. For instance, Alom et al. [26] suggest that deep learning (DL) should be used when the problem size is too vast for human reasoning capabilities, which is a fair description of the human immune system.

Ideally, an AI/ML model trained on clinical systems vaccinology data should be used to elucidate the key questions in vaccine mechanism of action. First, from training on available data, it should provide insights in common and distinctive features of vaccine technology platforms. Which immune processes are detectable for most or all vaccines, and which are characteristic for a specific technology? Are differences unambiguous, meaning do processes get turned on or off, or are the differences on the magnitude of the change? Second, can the model predict vaccine characteristics, such as efficacy, immunogenicity on antibody and T cell level, and safety and reactogenicity for the vaccines on which it was trained? Finally, when presented with new data, for instance novel immune signatures from a Phase I study with a new vaccine or vaccine platform, could an AI/ML model predict these characteristics?

However, despite this promise, AI/ML methods have not yet been applied to integration of systems vaccinology data across studies. Next, we will review challenges that hamper the application of AI/ML methods to systems vaccinology studies.

CHALLENGES FOR THE DEVELOPMENT OF AI/ML MODELS FOR CLINICAL SYSTEMS IMMUNOLOGY

As mentioned earlier, a recent publication by researchers from HIPC [19] describes in depth the challenges that need to be overcome to create a large, publicly available database of immune signatures that is consistent and comparable across studies [43]. They integrated data from 1405 participants in 53 cohorts covering 24 different vaccines. Whilst this is not a truly comprehensive capture of all clinical systems vaccinology studies, it is the largest public effort known to us. We therefore use it as the standard for this kind of effort. The group integrated transcriptional profiles and antibody response measurements and has made this data publicly available at: https://www.immunespace.org/, which is part of ImmPort [24], hosted by the National Institutes of Health (NIH). The researchers accounted for different gene expression profiling platforms, sample types, study design, age groups, vaccine type, target pathogen, and antibody measurements. They also highlight the differences in time points for the collection of the samples, which hampers the ability to develop comprehensive temporal models of vaccination.

In addition to highlighting key technical challenges of developing a database capturing these studies, the paper by Diray-Arce *et al.*

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[19] also provides an insight in other potential limiting factors of the available public domain data. Assessing the study characteristics provided by the authors, it shows certain interesting features. First, the database is heavily biased towards Influenza vaccine studies with 15 out of 33 studies (45%) being conducted with an inactivated Influenza vaccine. These studies account for 58% of the participants (718 out of 1228). The second most studied vaccine is the Yellow Fever vaccine that accounts for 12% of the studies and 8.7% of the participants, see Figure 1B for the distribution of the number of subjects per vaccine.

Second, the distribution of the number of participants across studies was skewed towards a small number of subjects per study. For instance, the median number of study participants was 24 per study. However, 36% of the studies had 13 or fewer subjects, with 7 studies (15%) having 5 or fewer subjects (Figure 1A).

Third, the immune response data was inconsistent as antibody titers, neutralization titers, or both were reported. T cell data were not included in the integration, neither was safety, reactogenicity or cytokine data.

Fourth, demographic information, such as, age, race, or ethnicity was not collected consistently.

Fifth, the data collected do not broadly cover available vaccine platforms. For instance, other vaccine classes, such as, nucleic acids (DNA and mRNA), adjuvanted, viral vectored, polysaccharide, and conjugate vaccines are missing or underrepresented.

Hagan et al. [20] performed a state-of-the-art analysis of the data captured in the ImmuneSpace database. In a large extent due to many of the issues described above, the authors relied on sophisticated statistical modeling approaches for their analysis. These factors together with the outlined technical challenges illustrate current limitations for using these data for AI/ML approaches. In short, the available data is not yet of sufficient quality to enable AI/ML application for integration of studies across published clinical systems vaccinology studies. Particularly, the black box nature of AI/ML models becomes a liability as its ability to capture and model complex features of the data might incorporate these imbalances and biases.

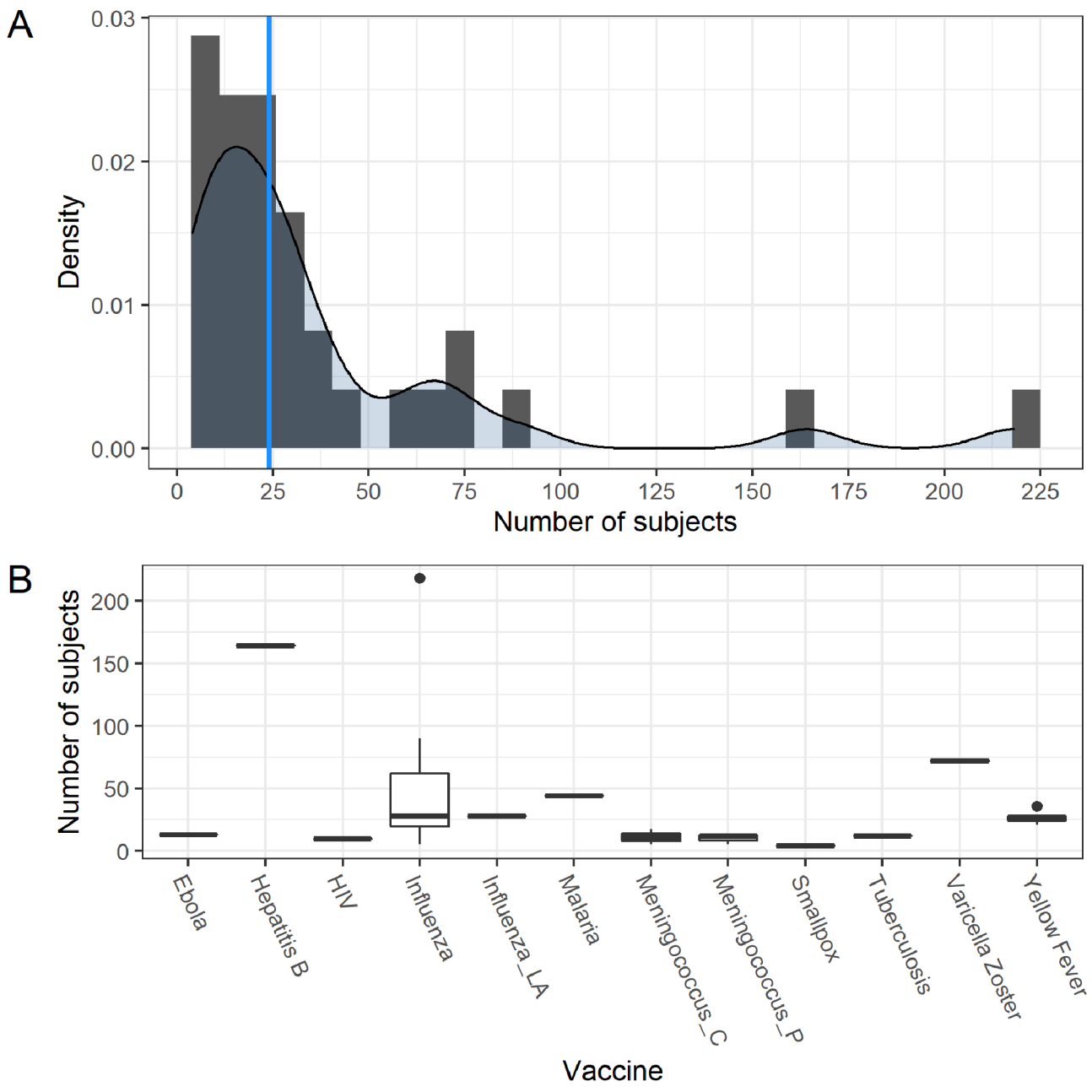
FUTURE DIRECTIONS

For developing AI/ML models for clinical systems vaccinology, we argue that the amount, type, and quality of data needs to grow further. To learn the hidden processes driving the complex immune response after vaccination and apply this knowledge to develop better vaccines, the experimental design of the studies can be further optimized. Typically, a study only covers one or two study arms, with a limited number of subjects per study arm. AI algorithms can more easily learn relevant information when they can compare responses over time in the controlled setting of a single clinical study. Especially learning about differences between and across vaccine platform technologies, would require comparisons with standard antigens [44, 45], or with different antigens delivered by the same platform.

Furthermore, untangling the immune response will require more comprehensive sampling of this response than what is usually done. In addition to transcriptomics samples, one would like to obtain measurements of cytokines, chemokines, and innate cell types, possibly at single cell resolution. As mentioned before, data on safety and reactogenicity is rarely reported, as are minimal demographics information, such as, age range, gender, or race. Moreover, better temporal resolution would be of added value. Various systems vaccinology studies, both human and animal, have shown that temporal dynamics can vary beyond the classical day 1, 3, and 7 post vaccination measurements [12, 45–47]. We also argue for better characterization of the adaptive responses. Antibody titers or neutralizing titers are often used as a proxy for efficacy; however, this is generally a simplification. For instance, in a controlled human malaria infection (CHMI) vaccine study in

FIGURE 1

Insight in study size of studies captured in ImmuneSpace.



A: Distribution of the number of subjects across the studies included in ImmuneSpace. The blue line indicates the median number of subjects per study. B: Boxplot of number of subjects per vaccine type. Ebola, HIV, and Tuberculosis are viral vectored vaccines; Hepatitis B and Influenza are inactivated, Influenza_LA, Smallpox, Varicella Zoster and Yellow Fever are live attenuated; Malaria is an adjuvanted protein, Meningococcus_C is conjugated, and Meningococcus_P is a polysaccharide vaccine. (Data from [19])

which a Ad35.CS.01, RTS,S/AS01, RTS,S/AS01 vaccine regime (ARR) was compared with the typical 3x RTS,S/AS01 (RRR), the vaccine efficacy was respectively 44 versus 52% [48]. In CHMI studies, antibody titers were often correlated with efficacy following challenge. Interestingly, the antibody titers of the protected subjects in the ARR arm were approximately at the same level as those for the unprotected subjects in the RRR arm, suggesting that antibody titers alone were insufficient to explain the difference. Indeed,

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both regimes induced differences in CD4 T-cells. Furthermore, additional research using CHMI studies showed that further exploration of the humoral response through systems serology [49] showed that specific antibody functionalities and the interplay with other parts of the immune system were important contributors to explaining protection against malaria challenge [50].

As shown in [19], integrating these diverse and exploratory readouts is complex and for many of these readouts there are typically no reporting standards for the data or the analysis methods, further limiting the perspective of obtaining such an integrated data set.

We envision that in the future AI/ML models can be applied to unravel the complexity of the human immune system in response to vaccination. However, as a community of systems vaccinologists, we still have a long way to go before we can bear the fruit of these methods.

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INTERVIEW

Embracing complexity

Charlotte Barker, Editor, *Vaccine Insights* speaks with **Gagandeep Kang,** Professor, Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India.

Pioneering enteric disease researcher Gagandeep Kang discusses her work on rotavirus vaccines, understanding immune responses to oral vaccines, and the importance of mentorship for the next generation of Indian health sciences researchers.

GAGANDEEP KANG is a Professor of Microbiology at the Wellcome Trust Research Laboratory, Division of Gastrointestinal Sciences at the Christian Medical College (CMC) in Vellore. She has worked on the development and use of vaccines for rotaviruses, cholera, and typhoid, conducting large studies to define burden, test vaccines, and measure their impact. Working in partnership with non-governmental organizations and the government, she has carried out phase I-III studies of rotaviral vaccines and provided laboratory support for vaccine development in India and for other developing countries. With the Indian Council for Medical Research and the World Health Organization, she has supported the establishment of networks of sentinel hospitals and laboratories that carry out surveillance for rotavirus disease in children and ancillary studies. She is the first woman working in India to be elected a Fellow of the Royal Society.

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Q What was your route to medical research?

GK: I started off wanting to be a doctor and then decided I wanted to be a microbiologist because I found infectious diseases so interesting. However, I soon found that the way microbiology was taught and done in India wasn't all that exciting, so I went into public health, and that's how I wound up studying vaccines in children in India.

Q What led you specifically to focus on gastrointestinal disease?

GK: I enjoy unraveling complexity. Infections can be in sterile or non-sterile sites in the body. In sterile sites, you usually identify the disease-causing pathogen quite easily. Non-sterile sites are much more complicated because when you see a bacteria or virus, you can't be sure if it is the cause of the symptoms or just a bystander. An added complexity with enteric infections is that most people have repeated exposures, and will respond differently to first, second, and subsequent exposures. That creates a situation with considerably more complexity, and I thought this would be an interesting area to study. If I had known just how much of a challenge it was going to be, I might not have been so brave!

> "Supplementing with both probiotics and zinc gives a marginally significant improvement in the immunogenicity of rotavirus vaccines."

Q Tell us about your work with rotavirus vaccines.

GK: I've been directly involved in developing four different rotavirus vaccines, as well as supporting the developers of two more. Of the four I was directly involved with, the ones that people generally talk about are the two that succeeded and are now WHO qualified – developed by Serum Institute of India and Bharat Biotech. But actually, two other vaccines never made it to licensure. One was a vaccine made by Shantha Biotechnics, similar to the Serum Institute of India vaccine that came later, and a heat-stable vaccine from Hilleman Laboratories. Both of those went into human clinical trials but ultimately did not progress.

I started out in the rotavirus field by looking for correlates of protection. We used state-of-the-art tools for investigating the immune response in Indian children, replicating a study conducted in Mexico in the late 1980s and early 1990s [1].

In the early 2000s, we established a very large study in India, around double the size of the Mexican cohort study, using a similar methodology [2]. To our surprise, we couldn't replicate the results. A long effort ensued to understand what was happening and confirm that rotavirus was behaving differently in India than it was in Mexico.

Based on this work, we predicted that vaccines would not perform well in low- and middle-income countries (LMICs). Soon afterward, the results of the first trials of the Merck and the GSK rotavirus vaccines conducted in Africa and Asia were released and proved our prediction correct, with both vaccines performing poorly in these settings. Soon after, we were similarly accurate in predicting the results of a Bharat Biotech candidate vaccine – we expected about 50% efficacy and saw 55% efficacy in the Phase 3 efficacy trial.

That led us to ask a larger question – why do oral vaccines perform poorly in LMICs? We've looked at polio and rotavirus vaccines, examining the influence of maternal antibodies, the microbiome, breast milk, and neonatal infections, and we've tried a variety of tactics to boost

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immune response, but so far, we've had limited success.

Supplementing with both probiotics and zinc gives a marginally significant improvement in the immunogenicity of rotavirus vaccines, which suggests that nutrition and the microbiome might make a difference. We also found that children who are neonatally infected have a much better immune response to the vaccine than children who did not acquire a neonatal infection, so another avenue is changing policies to include a neonatal dose of a novel rotavirus vaccine in low- and middle-income countries. But there is a lot more work needed to understand the complex factors underlying differences in responses to vaccines in low- and middle- versus high-income countries

"One of the most important lessons is that we need to trust our immune systems... We need to consider carefully who needs the boosters and when, and I don't think we have clear answers about the 'when' yet."

Q Your work is highly interdisciplinary – why is that?

GK: One of the unique points about doing medicine in a LMIC is that you don't necessarily treat just the disease – you treat the person and the community. When you think about the impact that rotavirus vaccines can have on children, their communities, and the country as a whole, you understand that you need to look beyond an individual child who has not responded to a vaccine and try to understand the reasons. That means you have to pull influence from other fields, ranging from studying gut function to doing health economics.

Q What are your top research priorities for the next five years?

GK: Right now, I'm working on typhoid and SARS-CoV-2. We are generating some really interesting data on SARS-CoV-2 infections and reinfections with the vaccines that we have in India. The next thing I want to take on is how to develop better tuberculosis vaccines, which is a huge challenge. It's interesting to note that a lot of what we learned from studying viruses and bacteria in the gut also applies to respiratory infections because they are also mucosal infections.

Q What lessons should we take from the COVID-19 pandemic?

GK: One of the most important lessons is that we need to trust our immune systems. If you're not elderly, and you don't have comorbidities, vaccines afford you really good protection against severe disease for a range of variants. I think people ignore that in a rush for still more vaccines and boosters. We need to consider carefully who needs the boosters and when, and I don't think we have clear answers about the 'when' yet.

Q You have previously commented that mentorship has not been prioritized in the Indian research culture. Do you still feel that's the case?

GK: A program called WomenLift Health has just started this year, which will allow a cohort of mid-career women in global health to be mentored by more senior scientists, me included. But these programs are still rare.

Of course, there are plenty of examples of senior scientists who support young people in institutions across India. I hope I am one of them – I measure myself not about the papers I publish or the research projects I complete but how many competent young scientists I leave behind to continue the work. However, there is a lack of systematic mentorship schemes, and given the size of India and the number of institutions we have, there are never enough mentors available.

The Indian system is hugely competitive because there are so many people wanting to progress and so few opportunities for them to grow. Along with competition comes hierarchy, with younger scientists expected to be hugely respectful to senior colleagues or teachers. Asking the professor detailed questions could see a student labeled disruptive or disrespectful.

I believe young scientists in India often don't recognize how good they really are and are not often given positive reinforcement, so I try to supply that. I believe we have the capacity – if we are given the opportunities – to do as well as anyone anywhere in the world.

Q What are the key strengths of Indian medical research?

GK: The fact that much of our work is grounded in where we are. We identify the problems that are truly relevant to us – and if they are relevant to us, they will have applications in other parts of the world. Few others can characterize those problems quite as well as we can, because we live with them every day.

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VACCINEINSIGHTS

PRECLINICAL & CLINICAL DEVELOPMENT

SPOTLIGHT

EXPERT INSIGHT

The quest for vaccine-induced immune correlates of protection against tuberculosis

Elisa Nemes, Andrew Fiore-Gartland, Cesar Boggiano, Margherita Coccia, Patricia D'Souza, Peter Gilbert, Ann Ginsberg, Ollivier Hyrien, Dominick Laddy, Karen Makar, M. Juliana McElrath, Lakshmi Ramachandra, Alexander C. Schmidt, Solmaz Shotorbani, Justine Sunshine, Georgia Tomaras, Wen-Han Yu, Thomas J. Scriba, Nicole Frahm; the BCG Correlates PIs Study Team & the M72 Correlates PIs Study Team

Immunization strategies against tuberculosis (TB) that confer better protection than neonatal vaccination with the 101-year-old Bacille Calmette-Guerin (BCG) are urgently needed to control the epidemic, but clinical development is hampered by a lack of established immune correlates of protection (CoPs). Two Phase 2b clinical trials offer the first opportunity to discover human CoPs against TB. Adolescent BCG re-vaccination showed partial protection against *Mycobacterium tuberculosis (Mtb)* infection, as measured by sustained IFNγ release assay (IGRA) conversion. Adult M72/AS01$_E$ vaccination showed partial protection against pulmonary TB. We describe two collaborative research programs to discover CoPs against TB and ensure rigorous, streamlined use of available samples, involving international immunology experts in TB and state-of-the-art technologies, sponsors, and funders. Hypotheses covering immune responses thought to be important in protection against TB have been defined and prioritized. A statistical framework to integrate the data analysis strategy was developed. Exploratory analyses will be performed to generate novel hypotheses.

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TB EPIDEMIOLOGY

Tuberculosis disease (TB) is caused by *Mycobacterium tuberculosis (Mtb)*, a mycobacterium transmitted by aerosol, which predominantly affects the lungs but can spread to any other organs [1]. Phylogenetic studies suggest that *Mtb* and humans co-evolved, possibly for the last 70,000 years [2]. For instance, TB may have killed up to 20% of the European and North American populations between the 17th and 19th centuries and was still responsible for the most annual deaths from a single pathogen globally until 2019 [3]. In 2020, global TB notification rates dropped to 5.8 million cases due to massive disruptions in testing and reporting caused by the COVID-19 pandemic, while the number of deaths increased, compared to 2019, to a total of 1.5 million [4].

Exposure to *Mtb* does not necessarily result in an established infection, but it is estimated that one-in-four humans are, or have been, infected with *Mtb* [5]. The population with viable *Mtb* infection is difficult to estimate since no diagnostic test can directly detect *Mtb* in healthy individuals. *Mtb* infection status is inferred from the measurement of immune sensitization to *Mtb* antigens by tuberculin skin tests (TST) or, more recently, IFNγ release assays (IGRA) [6]. Recent epidemiological models suggest that once *Mtb* infection is established, a large proportion of individuals may clear or effectively control infection, possibly associated with TST and/or IGRA reversion to negative, while a smaller proportion may progress to TB disease over their lifetime [7]. Progression to disease is thought to typically occur relatively rapidly, within two years of primary infection; late progression may be associated with re-infection or weakening of the immune response several years after primary infection.

Novel and improved diagnostics, treatments, and vaccines are urgently needed to address and ultimately end the TB epidemic [4]. Advocacy, political and economic commitments are also essential to address the common distorted perception that TB is an issue of the past, or that a deadly disease affecting mostly the poor is not a global emergency.

TB VACCINES

Bacille Calmette-Guerin (BCG) is the only available vaccine against TB [3]. This 101-year-old live, attenuated vaccine is routinely administered at birth in most countries [8] because it affords >80% protection against severe and disseminated TB disease, which has high mortality rates in children below 2 years of age [9]. Newborn BCG vaccination also confers partial protection against *Mtb* infection and pulmonary TB disease, but efficacy estimates vary greatly depending on age, geographical location, and previous sensitization to mycobacteria [9,10]. BCG saves lives, especially when given in early life, via both pathogen-specific and pathogen-agnostic immunity that is under intense mechanistic investigation [11,12], but has not stopped the TB epidemic; therefore, novel vaccines or immunization strategies are urgently needed.

While more efficacious and safer newborn BCG replacement vaccines are being tested to protect this vulnerable population, epidemiological modeling suggests that prevention of TB disease (POD) vaccines in adolescents and adults would have greater impact on TB transmission, and TB control in the general population [13]. Although the predicted impact of prevention of *Mtb* infection (POI) vaccines is lower compared to POD [14], measuring POI (or prevention of sustained infection, POSI) is also considered an innovative clinical trial end-point to efficiently provide proof-of-concept, since *Mtb* infection occurs much more often than TB disease [15]. However, PO(S)I vaccine efficacy may not necessarily predict POD, since immune responses required to prevent establishment of *Mtb* infection might be different from those required to prevent TB disease. Furthermore, partial prevention of infection may not translate to prevention of disease, since only a small minority of those who become

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infected typically progress to disease, and disease may preferentially occur in the subpopulation in whom infection occurred despite vaccination [1].

Rational design and clinical development of new TB vaccines have been hampered by a lack of immune correlates of protection (CoPs) [16]. In 2018, two Phase 2 randomized placebo-controlled clinical trials reported partial efficacy of novel TB vaccination strategies, which, for the first time, provide the opportunity to discover CoPs against established *Mtb* infection and TB disease.

In the first trial, which aimed to assess POI, BCG re-vaccination of IGRA-negative adolescents provided 45.4% (95% CI, 6.4 to 68.1) protection against sustained IGRA conversion, defined as conversion to a positive test without reversion to negative status 3 and 6 months post-conversion [17], which is suggestive of established *Mtb* infection. Immunogenicity analyses showed significant boosting of BCG-specific Th1 and Th22 cells, as well as modest induction of NK cell responses after re-vaccination [18]. Antibody responses to BCG were not measured in this trial.

In the second, a POD trial, vaccination of IGRA-positive adults with the investigational M72/AS01$_E$ vaccine provided 49.7% (95% CI, 2.1 to 74.2) protection against microbiologically confirmed pulmonary TB disease [19, 20]. Vaccination with M72/AS01$_E$ induced robust M72-specific IgG and Th1 cellular responses [20], as well as NK cell responses [21].

Key features of these trials that affect the design and potential outcomes of the CoPs analysis are summarized in Table 1.

TB IMMUNE CORRELATES PROGRAM

The TB Immune Correlates Program was initiated in 2018 for BCG-induced CoPs and in 2020 for M72/AS01$_E$-induced CoPs. The Program provides the strategy and governance structure to enable discovery of CoPs from established *Mtb* infection (inferred from sustained IGRA conversion in the BCG re-vaccination trial) and TB disease (microbiologically confirmed in the M72/AS01$_E$ trial) through a highly collaborative approach. The Program aims to: 1) Define hypotheses, biomarkers, and assays to be employed for the discovery of CoPs; 2) Develop the statistical framework and integrated data analysis strategy to evaluate the pre-specified hypotheses; 3) Pursue additional hypothesis-generating exploratory efforts; and 4) Ensure rigorous, efficient and streamlined use of the precious samples stored from these trials.

We hypothesize that vaccination induced a variety of immune responses comprising multiple immune cell subsets and effector mechanisms, which synergistically contributed to the control of *Mtb* growth following infection, resulting in reduced rates of sustained IGRA conversion (or increased rates of IGRA reversion) in the BCG trial, or reduced rates of TB disease (M72/AS01$_E$ trial) in vaccine compared to placebo recipients. Within this expectation, we aim to test a parsimonious set of pre-defined immunological hypotheses that are informed by the published literature, while allowing generation of additional hypotheses across a broad set of immunological compartments and mechanisms in a manner that rigorously controls the chance of false discovery. While the differences in trial designs and outcomes (Table 1) justify distinct hypotheses and experimental approaches, the overall alignment between the programs may enable identification of commonalities between the CoPs for POD and POSI.

Common between the two trials is the severe limitation of available samples (Table 1), particularly for cellular assays (peripheral blood mononuclear cells [PBMC]). To ensure feasibility and robustness, and to reduce the number of outcomes tested, it was deemed necessary to apply a staged approach, whereby pilot studies are conducted on a limited set of samples (excluding samples from participants who met the respective clinical endpoint [i.e., cases]) to down-select hypotheses and assays for the primary analysis comparing endpoint cases and non-cases. To preserve statistical

TABLE 1

Summary of BCG revaccination and M72/AS01$_E$ vaccine trials.

	BCG revaccination POSI trial	M72/AS01E POD trial
Intervention	BCG vaccine, 1 intradermal injection of $5x10^5$ CFU at Day 0	M72/AS01$_E$, 2 intra-muscular injections of 10μg M72
Formulation	Live attenuated *M. bovis* (Danish strain 1331), reconstituted in Sauton diluent without adjuvant, ~4000 antigens	Subunit vaccine (M72: recombinant fusion protein of Mtb32A and Mtb39A) with adjuvant (Adjuvant System containing MPL, QS-21 and liposome (25 μg MPL and 25 μg QS-21)
Population	659* HIV negative, IGRA negative adolescents, randomized 1:1 to BCG re-vaccination or placebo	3575 HIV negative, IGRA positive adults, randomized 1:1 to M72/AS01$_E$ vaccination or placebo
Efficacy	45.4 % (95% CI 6.4 to 68.1)	49.7 (95% CI 2.1 to 74.2)
Case Definition	Sustained IGRA conversion (secondary endpoint)	Culture or PCR-confirmed pulmonary TB without HIV (primary endpoint)
Endpoints	57 total 21/312 in BCG arm 36/310 in placebo arm	39 total 13/1626 in M72/AS01$_E$ arm 26/1663 in placebo arm
Vaccine-reactive immune responses in placebo arm	Detectable, high variability	Virtually undetectable, low variability
Samples for primary CoP analysis: PBMC Plasma Fixed cells from whole blood RNA from whole blood	 2 vials at day 0, 70, month 6 2 vials at day 0, 70, month 6 2 vials at day 0, 3 (placebo) or 7 (BCG), month 6 1 vial at day 0, 3 (placebo) or 7 (BCG), month 6	 6 vials at day 0, 37, month 6 2 vials at day 0, 37, month 6 2 vials at day 0, 37, month 6 1 vial at day 0, 37, month 6

*H4:IC31 recipients not included in CoP analyses.
CFU: Colony forming unit; MPL: 3-O-desacyl-4-monophosphoryl lipid A; QS-21: *Quillaja saponaria* Molina, fraction 21

power in the primary analyses, results from the pilot study will be used to select assays that exhibit the characteristics required of a CoPs biomarker (see below). Hypotheses will be pre-specified and prioritized in the primary analysis. Exploratory analyses will follow to generate novel hypotheses for validation in ongoing or planned larger trials of BCG re-vaccination and M72/AS01$_E$.

There are several key stakeholders of the TB Immune Correlates Program. Open calls-for-ideas were issued by the Leadership Team, separately for the BCG Program and the M72 Program. For both Programs, proposals from a large number of international investigators were evaluated by the Scientific Advisory Committee, and recommendations were made to the Funders, who further prioritized the most promising approaches to manage available resources. Established Biospecimen Governance Committees (including clinical trial sponsors and clinical site representatives) reviewed and approved sample access for the selected assays to the Principal Investigators (PIs) included in the BCG and M72 Correlates Study Groups. Results generated from the pilot studies will be evaluated and prioritized with a harmonized statistical approach, with analysis conducted by an independent statistical team [the Vaccines and Immunology Statistical Center (VISC) at Fred Hutchinson Cancer Center; Fiore-Gartland & Gilbert].

HYPOTHESES & EXPERIMENTAL APPROACH

Immune responses to *Mtb* are complex, they include many immune cell subsets and different effector mechanisms of the immune system and are affected by the extra-cellular milieu and pre-existing immunity [22,23]. Available evidence from pre-clinical models,

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particularly non-human primates (NHP), as well as cohort studies investigating immune correlates of risk for TB were considered to delineate the 'immunological space' to be covered by the pilot studies and to outline the primary hypotheses to be tested using several state-of-the-art technological approaches (Figure 1 & Table 2). Primary hypotheses and outcomes will be further refined based on results from the pilot studies.

CD4 T cells are considered the cornerstone of immunity against *Mtb*. Their antigen-specificity, functional and phenotypic profiles, differentiation and activation status, as well the capacity to home to the lung parenchyma are all considered key features of successful anti-*Mtb* immune responses. Two independent NHP studies using alternative BCG vaccination routes, mucosal or intra-venous, recently showed protection against *Mtb* infection and TB disease [24,25]. In both studies, increased abundance of mycobacteria-specific Th1/Th17 cells in the lung was associated with protection. No CoPs were identified in peripheral blood in the mucosal BCG study [24], while further experiments and analyses are ongoing in the intra-venous NHP model [26]. Even more recently, another NHP study showed that frequencies of T1/T17 and cytotoxic T cells measured within individual granulomas are associated with differential control of *Mtb* infection [27].

These data support the primary hypothesis that mycobacteria-specific CD4 T cells displaying a hybrid Th1/Th17 phenotype are the main mediators of a protective immune response to *Mtb*.

Antigen-specific T-cell responses will be measured primarily by intra-cellular cytokine staining and flow cytometry after antigen stimulation of PBMC from both trials [28].

For the M72 Program, additional approaches include sorting of M72-specific T cells and single-cell analyses including DNA-tagged antibodies, TCR sequencing, and RNA sequencing using different platforms. Since M72/AS01$_E$ only contains two *Mtb* antigens, further epitope mapping will be performed, as well as broader measurements of immunomodulatory factors secreted upon PBMC stimulation with M72 by multiplex protein detection assay.

Pathogen-specific antibodies are the primary CoPs for many effective vaccines, and multiple antibody functions beyond neutralization have been implicated in protection [29]. Studies conducted over a century ago showed some benefit of transferring serum from immunized horses to patients with TB, but antibodies to *Mtb* have not been consistently associated with protection (reviewed in [30–32]). Recent studies using modern techniques have reinvigorated the hypothesis that antibodies may play a role in mycobacterial control, predominantly through Fc-receptor (FcR)-mediated functionality that can lead to killing of *Mtb* in infected cells [33]. The postulated beneficial role of NK cells (reviewed in [34]) may well be linked to FcR-mediated effector functions.

These data support the co-primary hypothesis that antibody-dependent NK-cell activation contributes to control of *Mtb*.

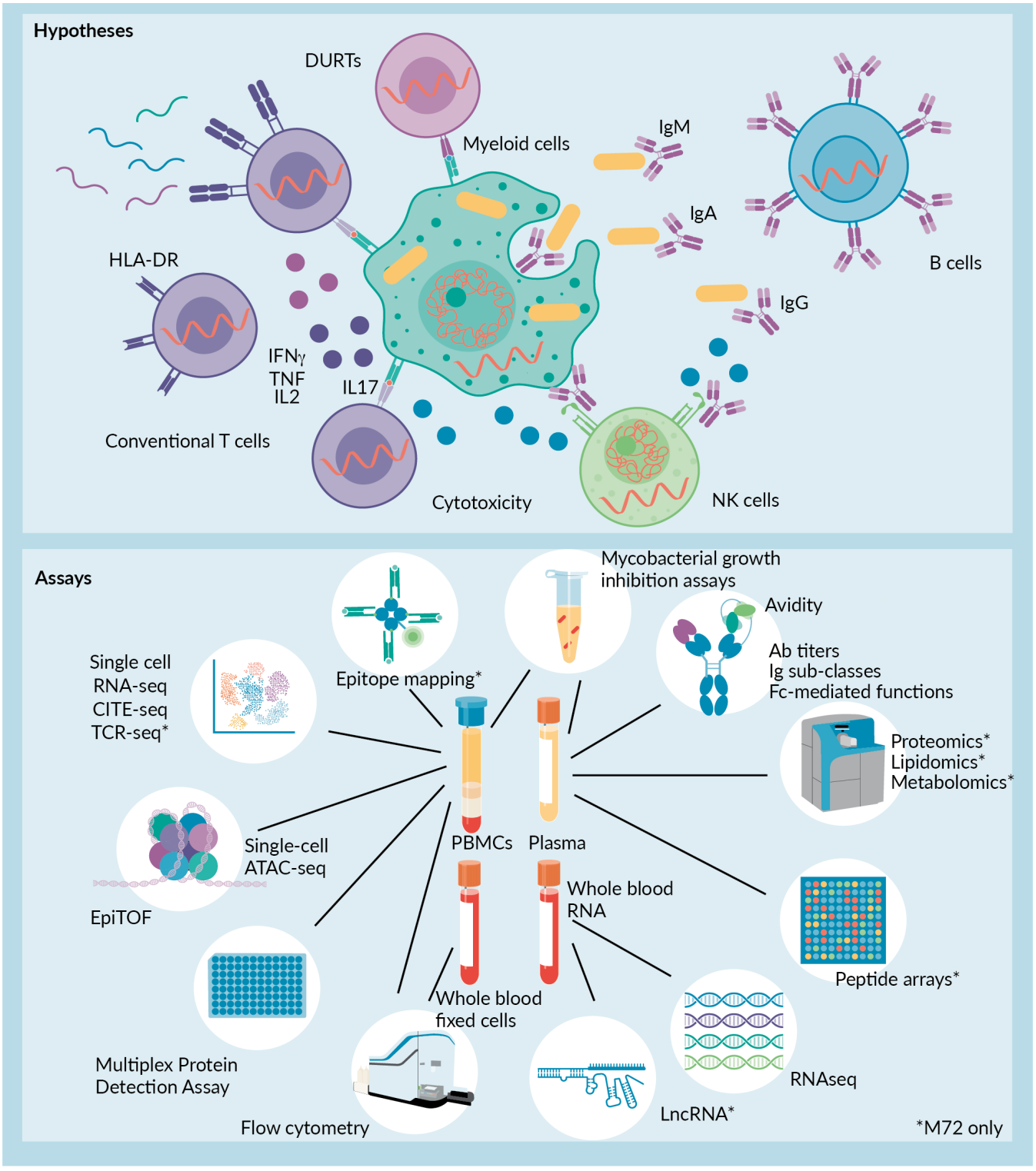
Antibody sub-classes may also be important for protection, presumably via a different mechanism to antibody-dependent NK-cell activation. An NHP study that assessed protection against *Mtb* following mucosal BCG vaccination suggested that mycobacteria-specific IgA responses in bronchoalveolar lavage may correlate with protection [24]. In a different study, robust IgM responses induced in the blood and lungs by intravenous BCG vaccination of NHP were associated with protection and reduced mycobacterial burden [26].

Antibodies will be profiled for both Programs using several approaches, which include measurement of titers, sub-classes, and avidity, as well as identification of Fc-mediated functions and antibody-mediated mycobacterial growth inhibition. Peptides recognized by antibodies will be identified by phage immunoprecipitation, peptide array, and *Mtb* proteome microarrays.

'Training' of monocytes/macrophages, and possibly NK cells, following innate immune activation by BCG or other pathogenic products has been well characterized [35] and has

FIGURE 1

Hypotheses and experimental approach.



Immune responses thought to be important for protection against TB have been identified and prioritized based on current knowledge. A systems immunology approach including several state-of-the-art assays will be employed to measure pre-selected outcomes as well as to generate new hypotheses (see text and Table 2 for more details). Most assays will be performed for both the BCG and M72/AS01$_E$ programs, those performed only for the M72/AS01$_E$ program are denoted by *.

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TABLE 2

Experimental approaches used to measure outcomes within each immune compartment.

Immune compartment	Outcome measure	Assay
Antigen-specific T-cell responses	Functional, activation and memory profiles	▶ PBMC-ICS and flow cytometry ▶ CITE-seq and Seq-Well S^3 or 10x Genomics on sorted T cells*
	Recognized epitopes	▶ Tetramer staining and flow cytometry* ▶ IFNγ ELISpot*
	TCR repertoire and gene expression	▶ Single-cell TCR sequencing on sorted cells (SMART-Seq2 or Seq-Well S^3 or 10x Genomics)* ▶ Immunoseq*
	Secretion of immunomodulatory factors	▶ Multiplex protein detection assay*
Humoral responses	Ab titers Ab sub-classes	▶ Binding Ab multiplex assay (BAMA)
	Ab avidity	▶ Biolayer Interferometry (BLI)
	Fc-mediated functions	▶ Ab-dependent NK cell activation ▶ Ab-dependent cellular phagocytosis ▶ Ab-dependent complement deposition ▶ Ab-dependent neutrophil phagocytosis ▶ Neutrophil extracellular traps ▶ Ab-depended dendritic cell phagocytosis ▶ Fc receptor binding array
	Ab specificity	▶ Linear peptide array* ▶ *Mtb* proteome microarrays* ▶ Phage immunoprecipitation*
Donor-unrestricted T cells	Functional, activation and memory profiles	▶ PBMC-ICS and flow cytometry
	TCR repertoire and gene expression	▶ Single-cell TCR sequencing on sorted cells (SMART-Seq2 or Seq-Well S^3 or 10x Genomics)* ▶ Immunoseq* ▶ CITE-seq and Seq-Well S^3 or 10x Genomics on sorted T cells*
	Phenotype and absolute counts	▶ Flow cytometry on fixed whole blood cells
Trained innate immunity	Epigenetic profiles	▶ EpiTOF (mass cytometry) ▶ Single-cell ATAC-seq ▶ Long non-coding RNA qPCR*
	Functional responses	▶ PBMC-ICS and flow cytometry ▶ CITE-seq and Seq-Well S^3 or 10x Genomics on bulk stimulated PBMC ▶ Secreted immunomodulatory factors in response to heterologous stimuli (O-Link)
Cooperation between immune compartments	Mycobacterial growth inhibition	▶ Heterologous macrophage MGIA with autologous plasma ▶ Heterologous whole blood MGIA with autologous plasma ▶ Autologous PBMC and plasma MGIA*
Innate immunity / milieu	Bulk PBMC functional, activation and memory profiles	▶ CITE-seq and Seq-Well S^3 or 10x Genomics* on bulk stimulated PBMC
	Immunophenotype and absolute counts	▶ Flow cytometry on fixed whole blood cells
	Gene expression profiles and transcriptomic TB signatures	▶ RNA sequencing on whole blood
	Apolipoproteins and complement	▶ Targeted LC/MS*
	Lipidomics	▶ LC-MS/MS*
	Proteomics	▶ LC-MS/MS*
	Metabolomics	▶ GC-MS*

*M72 only. Ab: Antibody; GC-MS: Gas chromatography and mass spectrometry; ICS: Intra-cellular cytokine staining; LC/MS: Liquid chromatography and mass spectrometry; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; MGIA: Mycobacterial growth inhibition assay; PBMC: Peripheral blood mononuclear cells; TCR: T cell receptor.

been associated with broad pathogen-agnostic protection against unrelated infections in humans [36]. AS01, the adjuvant used in the M72 vaccine, is known to potently activate the immune system [37]. It must, however, be acknowledged that trained immunity, as currently understood, may not be sufficiently long-lived to explain the durability of protection, which was observed up to 2 years after BCG re-vaccination and up to 3 years after M72/AS01$_E$ vaccination.

Nevertheless, investigation of the role of innate – including trained – immunity is warranted considering its possible role in inducing and supporting the development of long-lasting adaptive immune responses.

Since epigenetic re-programming is a key feature underlying trained immunity, it will be measured by EpiTOF [38] and single-cell ATAC-seq. Immune-gene priming long non-coding RNAs regulate the deposition of H3K4me3 at the promoters of immune genes [39] and will be measured by qPCR. Functional responses to heterologous stimuli will be measured by O-Link in the supernatant of stimulated PBMC in both trials.

Technological advances have also contributed to a much more refined understanding of non-classical, so-called donor-unrestricted T (DURT) cells, which recognize non-protein-based antigens, and their role in mycobacterial control [40]. It was recently shown that the frequencies of DURT cells were not modulated by primary vaccination or re-vaccination with BCG [41]; however, the effects of BCG re-vaccination on their functional or phenotypic attributes have not yet been fully explored. Presentation not only of peptides but also non-protein-based antigens by the non-polymorphic antigen-presenting molecules CD1, MR1, and HLA-E may contribute to the pool of protective mycobacterial T-cell responses induced by BCG re-vaccination. While clear correlations with *Mtb* infection have not been described for these T-cell subsets, enrichment of mucosa-associated invariant T (MAIT) cells has been observed in exposed individuals who remain uninfected [42], supporting the hypothesis that BCG-induced DURTs contribute to the early control of *Mtb* infection.

DURT cell frequencies and absolute counts will be measured in fixed whole blood by flow cytometry and their function by intra-cellular cytokine staining after PBMC stimulation.

In addition to generating outcome measures related to our primary biological hypotheses, several assays will be employed that provide an unbiased view of vaccine-induced changes and are thus likely to generate new hypotheses, or define the systemic milieu in which immune responses are induced by vaccination.

A mycobacterial growth inhibition assay [43,44] will be used as a functional readout to determine whether M72/AS01$_E$ vaccination enhances overall mycobacterial growth control or even killing in vitro, as well as the relative contribution of cell-mediated and antibody-mediated immunity to this outcome.

Another hypothesis is that RNA sequencing (RNA-seq) analysis of whole blood, as well as single cells, will identify novel gene expression profiles, cellular subsets, or pathways associated with protection against infection and/or disease. The whole blood RNA-seq dataset from the M72/AS01$_E$ trial will also be mined to test the hypotheses that blood transcriptomic signatures of risk of TB, which allow identification of individuals in early stages of disease progression, or with subclinical disease [45–47], are elevated in M72 trial participants who developed TB within the first year [47] compared to non-cases. Further, blood transcriptomic signatures associated with protection identified in animal models [48,49] will be assessed for increased expression in non-cases compared to cases.

To test the hypothesis that vaccination with M72/AS01$_E$ elicits a multimolecular biosignature in participant plasma correlating with protection from active TB [50–52], lipidomics, metabolomics, and proteomics approaches as well as targeted measurements of apolipoproteins and complement proteins will be undertaken.

Finally, phenotyping of whole blood leucocyte populations provides a snapshot of

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immune status in the absence of stimulation. Baseline expression of the activation marker HLA-DR on T cells [53], increased myeloid to lymphoid cell ratio [54] and reduced abundance of NK cells [55] have all been associated with increased risk of TB and will be assessed as biomarkers of risk of TB, or as CoPs. Whole blood immunophenotyping and absolute counts will be performed by flow cytometry [56].

STATISTICAL CONSIDERATIONS

The primary objective of the statistical analyses for the Pilot Study is to describe and rank assay readouts based on their potential to predict sustained infection or TB disease in future case-cohort analyses. Participants were selected for the Pilot Study at random from among the vaccine and placebo recipients that did not meet any of the trial endpoint criteria. Each lab will analyze a baseline and post-immunization (BCG: Day 70 post-BCG; M72/AS01$_E$: Day 37, 1-week post-2nd injection) sample from a subset of vaccine (n=64) and placebo (n=22) recipients.

A head-to-head analysis will be conducted by the statistical team to quantify vaccine-induced immune responses, characterize performances of the assays, and identify a set of low-dimensional biomarkers. These analyses will inform decisions about which assays to prioritize for the Primary analysis of cases and non-cases.

Assay and readout performance will be evaluated using the following criteria:

- Broad, biologically-relevant dynamic range among vaccine recipients after vaccination
- Broad, biologically-relevant dynamic range among all participants at baseline
- Low intra-individual temporal variability among placebo recipients
- Large shift in the distribution among vaccine vs. placebo recipients (i.e., evidence of a vaccine-induced response)
- Low technical measurement error
- Low covariation among readouts within each assay, with a low-dimensional representation of the measured response
- Low covariation of readouts across assays, reducing overlap and redundancy in immunological space
- Broad coverage of relevant immune functions

With these criteria, we will attempt to deconstruct the variance of each readout into the components of vaccine-induced and non-vaccine-induced variation. We will also seek to select assays and readouts that maximize the proportion of variability that could possibly correlate with TB risk or vaccine protection (Figure 2).

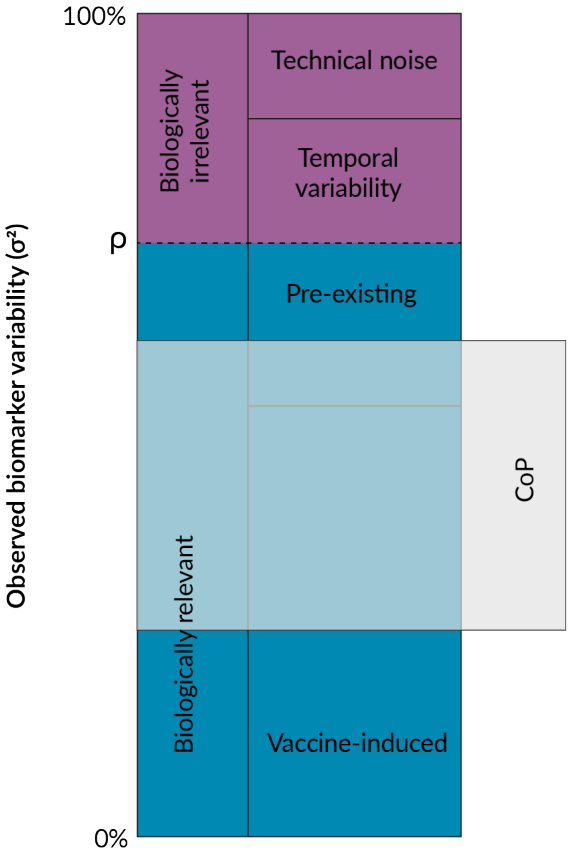
The sample size required to evaluate these outcomes in the Pilot studies, considering the expected variability of baseline BCG- and M72-specific responses as well as the number of cases available for the final analysis of each trial (Table 1), was determined to be 24 BCG and 12 placebo recipients for the BCG Program, and 40 M72/AS01$_E$ and 10 placebo recipients for the M72 Program [57].

The goal of the Primary statistical analysis will be to evaluate each biomarker and combinations of biomarkers as correlates of risk (CoRs) and CoPs using a 'case-cohort' design, including cases and non-cases from the randomized vaccine and placebo treatment groups.

For the BCG Program, two different case-cohort analyses will be performed (Figure 3). In the 'modified intent-to-treat (mITT)-controlled analysis' sustained IGRA converters will be compared to participants who did not display sustained IGRA conversion, i.e., a combination of participants who remained IGRA-negative throughout the study and those who showed initial IGRA conversion, but subsequently reverted to IGRA-negative ('IGRA reverters'). In the 'reversion-controlled analysis' sustained IGRA converters will be compared to IGRA

FIGURE 2

Partitioning of biomarker variability.



The inter-vaccinee variance of each biomarker is made up of biologically relevant and irrelevant components. Irrelevant components contain measurement error and types of temporal variability that cannot be correlated with protection; measurement error can be estimated from technical replicates while temporal variability can be estimated from longitudinal sampling of placebos. Pre-existing and vaccine-induced variability in the marker can both be correlated with protection. The biologically relevant proportion of variation (ρ) can be affected by pre-existing factors like host-genetics (e.g., HLA, TLR SNPs), microbiome, or pre-existing immunity to *Mtb*.

reverters only. The all mITT-controlled analysis includes participants who remained IGRA negative throughout the study, who likely include a combination of non-exposed individuals as well as exposed individuals in whom vaccine-induced responses or natural immunity were able to prevent initial infection. The latter group is impossible to unequivocally identify in this study since exposure (e.g., to household members with active disease) was not measured for participants. The reversion-controlled analysis on the other hand includes subjects who were clearly exposed since they showed initial IGRA conversion, but then presumably were able to control infection, allowing them to revert to IGRA negative. This analysis therefore controls for exposure, although it does exclude exposed participants who do not convert. In both types of analysis, immune markers that are enriched in vaccine recipients who do not become sustained converters may be identified as putative CoPs. The total number of participants in each of the groups is shown in Figure 3 (n=57 cases and n=187 non-cases).

For the M72/AS01$_E$ Program, all cases (n=39) meeting the primary TB disease endpoint definition will be included [20]. A higher non-case-to-case ratio of samples has been proposed for the M72 study (5:1, n=195 non-cases) compared to the BCG study (3:1) to increase statistical power [58] and adapt for the fewer number of cases in the M72 study (Table 1). Since the BCG study was conducted at two clinical sites in the same province in South Africa [59], no site-specific considerations were required for the selection of non-cases. However, the M72/AS01$_E$ trial was conducted at 11 clinical sites across three African countries and significant differences in immunogenicity were noted when comparing participants recruited in South Africa and Kenya [20]. The recruitment site will therefore be an important variable to consider in the selection of non-cases.

CoPs will be evaluated using a range of statistical methods including covariate-adjusted regression to evaluate correlates of risk within each treatment group, a vaccine efficacy modification or 'principal stratification' framework to estimate vaccine efficacy as a function of the vaccine-induced biomarker [57,60], a causal mediation framework to estimate the proportion of the efficacy that can be explained by the biomarker [61] and a machine learning framework, which frames the analysis as a multivariate classification of cases vs. non-cases [62]. Analyses will leverage existing code that was developed and implemented for studying CoRs and CoPs in the US government COVID-19 vaccine trials, including an open-source statistical analysis

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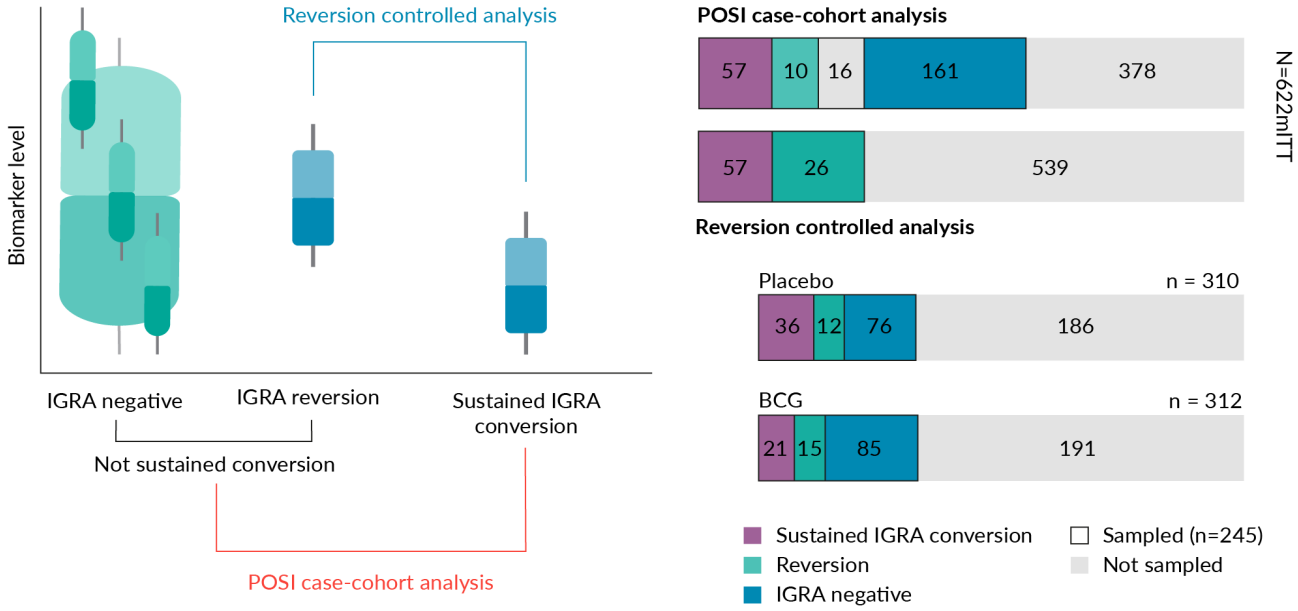
FIGURE 3

Sampling strategy for the BCG re-vaccination prevention of sustained infection (POSI) case-cohort immune correlates study.



The correlates analysis will include two distinct comparisons: (1) Sustained IGRA conversion vs. no sustained conversion (POSI Case-Cohort Analysis), and (2) Sustained IGRA conversion vs. IGRA reversion (Reversion Controlled Analysis). The POSI Case-Cohort Analysis samples controls from participants without a sustained IGRA conversion, including a mixture of potentially exposed, unexposed and IGRA converted-reverted. In contrast, the Reversion Controlled Analysis conditions on initial conversion, excluding unexposed individuals and focusing on the phenotypic differences that distinguish reversion from sustained infection.

plan and open-source codebase [63]. Similarly, all Primary analyses will be pre-specified in a statistical analysis plan [64].

Exploratory analyses will also be conducted to evaluate a broader set of potential biomarkers and to infer mechanistic insights from their attributions to protective immunity. With multi-scale cross-cell type 'omics' data generated in the case-cohort studies, an integrative multivariate framework that directly models data from several platforms will provide an insightful view of the cross-talk between immune cell types while simultaneously identifying new candidate biomarkers. Specifically, supervised integration models will identify a set of biomarkers that maximize the covariance with phenotypic outcomes while considering interactions among multiple data modalities. In addition, a module-based method transforming genes/proteins/metabolites into pathways/cellular responses based on prior biological knowledge will be integrated into the multivariate modeling to improve data interpretation. As there are many new emerging multi-omic predictive algorithms [65,66], we are benchmarking existing algorithms holding robust performance and applicable to multi-scale cross-cell type data modalities. Moreover, network analyses through partial correlation networks or Gaussian/mixed graphical models from data integration will also be performed to infer potential functional linkages between immune responses and vaccine efficacy. These descriptive analyses will generate novel hypotheses that may be evaluated in future studies.

PROGRESS & HURDLES

The BCG Program was launched in late 2018, pilot studies have been completed, and data review is expected in October 2022, followed by a swift selection of the assays that will be included for the Primary analysis,

which should be completed in 2023. The M72/AS01$_E$ CoPs Program was launched in early 2020 and Pilot studies are expected to start in late 2022.

Both TB Immune Correlates Programs have been heavily affected by disruptions caused by the COVID-19 pandemic, including closure of laboratories in 2020 and general institutional de-prioritization of non-COVID-related research. Additionally, recent changes in South African data privacy legislation (the Protection of Personal Information Act [POPI Act], https://popia.co.za/) resulted in significant delays in the fulfillment of regulatory and contractual requirements due to the unfamiliarity of participating research institutions with the requirements set forth in the act.

The decision to issue open calls for proposals and to establish two large consortia of international investigators was made to ensure that the best possible scientific expertise and state-of-the-art technologies were deployed in the fight against TB, with a spirit of collaboration and data sharing. The hurdles in fulfilling regulatory and contractual requirements involving multiple partners in a timely fashion were initially under-estimated and resulted in significant delays.

CONCLUDING REMARKS

The year 2018 was dubbed 'the year of TB vaccines' reflecting the publication of vaccine efficacy results for BCG revaccination (POSI) and M72/AS01$_E$ (POD). The scientific community has been waiting in hopeful anticipation for the identification of immune correlates of protection against TB, which can be discovered now that randomized placebo-controlled clinical trials of partially efficacious vaccines have been completed (Box 1). The COVID-19 pandemic has greatly affected TB patient management and research programs around the world and caused much frustration within the TB research community, which has been chronically under-funded and de-prioritized, despite focusing on the infectious disease that has killed most humans (and continues to do so) in history. The speed and success of COVID-19 research is inspiring, and provides new hope that when vaccine developers, funders, and scientists establish collaborative partnerships with strong political and public support the unimaginable (several COVID-19 vaccines developed at warp speed and identification of the first correlates of protection months thereafter) can happen [63,67]. This sense of urgency and scale now needs to be applied to TB vaccines.

BOX 1

Translation insight

A CoP for one or both of these TB vaccines has enormous potential to accelerate vaccine development and the clinical development pathway. A 'mechanistic CoP' [68], established by these studies and validated in follow-up experiments, can inform the field about the roles of host immune responses in *Mtb* infection and disease progression, thereby facilitating iterative vaccine design and prioritization of vaccines in the clinical pipeline. However, a CoP does not need to be mechanistic to be clinically valuable; a validated statistical CoP – with known or unknown protective mechanisms - can be used to de-risk clinical development and accelerate vaccine licensure through 'immuno-bridging'. With a validated CoP, a vaccine could even be licensed on the basis of meeting specific immunogenicity criteria developed from CoPs studies of a previously licensed vaccine [69]. Therefore, establishing a CoP for PO(S)I or POD based on the BCG and M72 studies could have implications for TB vaccine development well beyond these two vaccines. Candidate CoPs identified by the studies described here will require independent validation, which is possible by leveraging a larger BCG re-vaccination POSI trial ongoing in South Africa (NCT04152161) and a Phase 3 trial with M72/AS01$_E$, which is currently being planned.

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VACCINEINSIGHTS

PRECLINICAL & CLINICAL DEVELOPMENT

SPOTLIGHT

Strengthening regulatory oversight & boosting clinical trial capacity in Africa

Bartholomew Dicky Akanmori
World Health Organisation

"AVAREF should be recognized as a regional platform that offers developers the opportunity to conduct clinical trials to international standards."

VIEWPOINT

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On July 5, 2022, Charlotte Barker, Editor, *Vaccine Insights*, spoke to Dicky Akanmori about building clinical trial capacity in Africa. This article has been written based on that interview.

An increasing number of vaccine clinical trials have been carried out in Africa over the last 20 years. Through initiatives like the African Vaccine Regulatory Forum, clinical trial capacity has increased greatly – but we must not stop here. Continuing to build clinical trial capacity must be recognized as a critical goal for African nations.

Around two decades ago, African nations began to gain prominence as sites for clinical trials of important drug products, particularly vaccines against priority diseases such as TB, HIV, and malaria. Now, Africa is one of the preferred places to carry out trials for many drugs. One reason is that the burden of many important communicable diseases is high on the continent, allowing developers to arrive quickly at their endpoints. Other diseases are unique to Africa, making it the only place trials can be carried out.

However, while more and more clinical trials were being run in Africa, the systems for regulating these studies in human subjects were not adequate in many cases, with some countries lacking even the legal framework needed to ensure that participants are treated ethically and studies are informative. Inconsistencies between different countries and low-quality reviews led to cases of 'approval shopping'.

In 2006, WHO established a project to build capacity for the regulatory oversight of clinical trials in Africa, through a network approach. Driven by scientific evidence, the African Vaccine Regulatory Forum (AVAREF) harmonizes standards and regulatory processes and builds capacity for regulatory oversight of clinical trials across the African continent. Specifically, AVAREF aims to:

1. Establish legal frameworks to govern clinical trials on the continent, for both ethics and regulatory authorities.
2. Address the disparities in the systems of processes in different countries for a more harmonized approach.
3. Deal with the long timelines (sometimes years) for receiving decisions following the submission of clinical trial protocols.

One of the first activities organized by the member countries was a joint review of a vaccine study that was planned to take place in several African countries (MenAfriVac). By pooling capacities, we ensured an effective review and helped set in place processes that all regulators can use, allowing easier exchange of data in the future. Joint reviews – and joint inspections – are now the mainstay of AVAREF's activities.

Another important activity is the development of guidelines, templates, and additional tools required for the successful review of clinical trial submissions. A standard format for submissions was developed and is now used in several African countries. This has benefited ethics committees and regulators and has also been a tremendous help to clinical trial sponsors on the continent, who now know that there is one standard application format that will ensure the application is complete.

Progress has been made. Four countries in Africa have now reached maturity level 3 based on assessment using the WHO's global benchmarking tool, meaning they effectively have regulatory systems capable of providing oversight for clinical trials which meets international standards. Formerly, none met this standard.

However, despite these improvements and others, clinical trial capacity in Africa is still not adequate. Regulatory timelines have been reduced, and countries have better processes in place, but the capacity remains relatively weak compared to high-income countries. One reason is that Investment in R&D is still very low – for every $100 spent on R&D in high-income countries, only $1 is spent in Africa.

Capacity is also driven by the number of scientists, regulators, doctors, and nurses available to plan and run clinical trials. Even in high-income countries this can be a challenge, but in Africa, there are no nurses or doctors to spare for clinical trials – they are needed for frontline services.

The COVID-19 pandemic exposed important weaknesses in health system infrastructure, from laboratories to digital records,

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which limit the capacity for clinical trials. But COVID also presented opportunities. The pandemic pushed countries to implement better processes, including digital systems for clinical trials, product registration, and safety monitoring. It also taught African nations that they can rely on one another – if one country doesn't have sufficient capacity, it can join forces with others.

In my view, it is critical that we improve clinical trial capacity across the continent. Product development can no longer be limited to high-income countries, especially when those products are destined to be used in lower- or middle-income countries, as has been the pattern over decades and centuries.

How can we improve capacity? First, we need a broad stakeholder consensus to focus on clinical trial capacity in Africa. Even if it does not translate immediately to direct funding, the recognition – from product developers, sponsors, funders, philanthropic organizations, and international public agencies – that Africa remains vital to product development is important and could have longer-term support.

Not only would a focus on building clinical trial capacity in Africa address public health goals, by making sure that products can be developed for the priority diseases of the continent, but it also has the potential to open up new markets for manufacturing health products in Africa. COVID-19 vaccines are a good example of a situation when – even once vaccines became available, manufacturing facilities could not cope with the demand, and high-income countries bought up much of the initial limited supplies. If we had a broader capacity for manufacturing these products around the globe, faster and more equitable distribution of vaccines would have been easier.

Second, AVAREF should be recognized as a regional platform that offers developers the opportunity to conduct clinical trials to international standards.

If these two things were achieved, the world would be on the right path to addressing the inequities that we saw with respect to COVID-19 vaccines.

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VACCINEINSIGHTS

PRECLINICAL & CLINICAL DEVELOPMENT

SPOTLIGHT

COMMENTARY

EATRIS: providing the right tools, at the right time, for vaccine development in a pandemic

David Morrow, Lucia Gabriele, Antonio L Andreu, Florence Bietrix, Anton Ussi & Jan Langermans

As society continues to take its first cautious steps out of the COVID-19 pandemic, researchers, including vaccine developers, continue to reflect on the challenges that they faced and overcame together during the last 2 years and the improvements necessary for better pandemic preparedness in future. Now more than ever, the world is recognizing the importance of vaccines, and the European Research Infrastructure for Translational Medicine (EATRIS) and its infrastructure partners across Europe and beyond have strived to accelerate promising candidates through the R&D pipeline, by offering innovative services to vaccine developers in academic and industry settings.

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Removing traditional boundaries to accessing key technologies in a fast and efficient manner has been central to our approach to facilitating best practice vaccine development. Combining critical preclinical and clinical services and expertise including regulatory guidance, across EATRIS and different research infrastructures, and providing rapid access to these resources, has always been a key strategy for EU research

infrastructures. The ability of individual and combined research infrastructures to provide the right tools for vaccine developers has proved essential in the face of this recent global health emergency and will be even more so for future pandemics.

EATRIS COVID-19 RESEARCH FORUM

EATRIS's core mission is to accelerate the translation of promising scientific discoveries into benefits for patients [1–5]. Its focus is to undertake and facilitate activities that bridge the innovation gap between the lab and the clinic by offering services and expertise to increase the chances of cutting-edge research successfully reaching the patients that need it most. From the very start of the pandemic, our EATRIS community, composed of researchers from over 120 university medical centers and research facilities across 14 EU countries, placed themselves and their expertise at the forefront of the COVID-19 fight.

The immediate question for EATRIS was how best to support them and capitalize on their activities for advancing research in the field? Our first task was closely monitoring the efforts undertaken by our EATRIS members to create a comprehensive but by no means exhaustive list of relevant activities that they engaged in and, additionally, the essential services they could provide for COVID-19 research for vaccine and anti-viral developers. EATRIS sites across Europe began populating this list of available COVID-19 resources and their relevant activities, such as ongoing clinical trials, and made it publicly available on the EATRIS website to internal and external researchers.

The aim of this activity was simple – to support researchers in their vaccine, therapeutic or diagnostic development, with the best possible technologies, research tools, and expertise. As the list grew, these institutions formed the EATRIS COVID-19 Research Forum, where any service requests or need for specific expertise from academia, industry, or governmental group related to COVID-19, including vaccine development tools and regulatory guidance, matched specifically within this group. To expedite the process, the need for contracting and facilitation fees was removed, and both client and service provider were connected within 48 hours. This rapid response COVID-19 service was developed into an online platform where all resources and activities could be shared, in addition to relevant news items and resources of interest to the COVID-19 researcher such as data portals, and relevant animal models and their availability.

Central to this list of resources was and continues to be a world-class, broad range of consolidated know-how and resources across our Institutions that support the vaccine and immunotherapy developer. This includes a dynamic and harmonized flow of knowledge and expertise joining standardized, validated, and innovative technologies for the investigation, characterization, and monitoring of the immune and inflammatory network and responses in vaccine and therapeutic development. This includes critical immune monitoring and profiling services for the vaccine developer at various developmental stages including:

- Systems-level characterization of immune cells in human tissues
- Epigenetics of immune cells
- Access to tissues explant models to evaluate the role of individual immune subsets against infectious diseases and to characterize viral isolates
- Functional studies of pathogenicity of genetic variants
- Virus neutralization testing
- Immune profiling in different animal models
- Virus-specific immune responses
- Quantification of immunological subsets.

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This knowledge and service provision continues to strive to meet the needs of biotechnology companies, the pharmaceutical industry, and the academic research community developing vaccine candidates. In addition to listing these must-have technologies and services, the forum included funding calls, publications, and open research service requests to which members could apply or respond. By the end of 2020, the EATRIS Covid-19 Research Forum consisted of over 90 active researchers across 43 institutions from 14 EU countries. Although similar supportive initiatives exist across different networks and infrastructures, the simplicity and flexibility of this forum, including a willingness to support each other in a fast and efficient manner, was a hallmark of its success and continues to drive its utility.

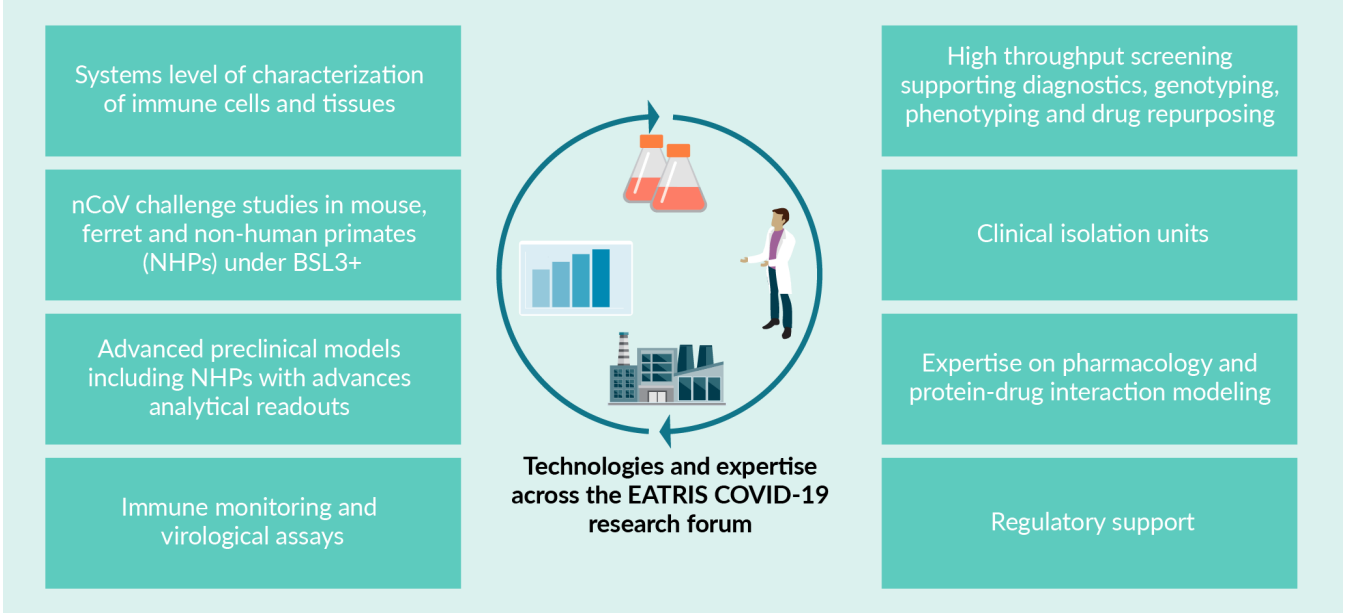
As a direct result of the EATRIS COVID-19 Research Forum Group, over 50 projects have been facilitated within this group to date. The establishment of the EATRIS COVID-19 Research Forum Group in 2020 represents a strong example of how our network of institutions within the infrastructure can pool their resources together to create an efficient array of must-have services for novel vaccine and therapy development in a time of urgent need. As a result, we must look ahead with a strong commitment to further improving this initiative, with the single aim of supporting our researchers and other infectious disease experts to the best of our abilities. A sample of these resources, including preclinical and clinical tools, are listed in Figure 1 and continue to prove essential in facilitating vaccine development.

COMBINING TOOLS & EXPERTISE ACROSS RESEARCH INFRASTRUCTURES

EATRIS's mission is highly complementary to several other research infrastructures, including those of the European Clinical Research Infrastructure Network (ECRIN), and the European Research Infrastructure for Biobanking (BBMRI). The COVID-19 Fast Response Service was established at the start of the pandemic and is still an active output of our strategy, representing a coordinated and accelerated procedure for researchers to access

FIGURE 1

Core technologies and expertise across the EATRIS COVID-19 research forum.



the academic facilities, services, and resources of the three medical research infrastructures [6]. This example of research infrastructures working together under the umbrella of the European Alliance of Medical Research Infrastructures ("EU-AMRI") allows vaccine developers to draw on resources, including pre-clinical and clinical tools and expertise, where and when needed through one access point. Similarly, TRANSVAC [7], an EU-funded project of which EATRIS is a partner with several other infrastructures and leading vaccine developing institutions across the EU, has a central aim to accelerate vaccine candidates across the R&D pipeline, by offering services to vaccine developers in academic and industry settings. Researchers developing vaccine candidates against COVID-19 and other infectious diseases have benefitted and continue to benefit from TRANSVAC services, including non-clinical in vivo models, adjuvant formulation, clinical trial and regulatory support, and others. Future calls for vaccine development services are due in the coming months, which developers can continue to apply for through the TRANSVAC website.

Different research infrastructures bring their own resources and expertise to vaccine development where available and have all offered their own valuable programs to facilitate vaccine development during this pandemic [8]. Real benefit, however, is provided to vaccine and therapy developers, when relevant biomedical research infrastructures combine their catalogs of preclinical and clinical tools. This offers the vaccine developer the entire spectrum of technologies they require to further develop their innovative vaccine candidates.

EATRIS is now working together with multiple research infrastructures, including over 154 partners across 34 countries, which has assembled the largest and most diverse research- and service-providing instrument to study infectious diseases in Europe. This project, "Integrated Services for Infectious Disease Outbreak Research" (ISIDORe) was granted funding of 21 million Euros under the Horizon Europe funding program and launched on February 24, 2022. It aims to improve Europe's global service and research capacities in the face of a future pandemic. ISIDORe is an interdisciplinary project coordinated by ERINHA (the Research Infrastructure dedicated to the study of high-consequence emerging and re-emerging pathogens) and brings together all key European life-sciences research infrastructures and networks, as well as those in the social sciences [9]. The Consortium brings together infrastructure partners under the umbrella of 17 different partners (Figure 2), including 14 research infrastructures. Collectively, the vaccine developer can access upon application, free services provided from each of these infrastructures which covers the entire pipeline of vaccine development.

The ambition of ISIDORe is to provide fast access to these cutting-edge resources to scientific user communities for supporting their evidence-based development or adoption of countermeasures. This platform is designed to be further expanded to include infrastructures from across the globe to combine strengths and to help remove the gaps in the translational science pipeline to support diagnostic, therapeutic, and vaccine development during a global health emergency. ISIDORe will contribute to Europe's readiness for any epidemic-prone pathogen through a global, integrated, and preparedness-driven approach. It will provide free of charge access to innovative resources and services to scientific user communities for supporting their research projects in the field of infectious diseases.

PANDEMIC PREPAREDNESS: LESSONS LEARNED FROM ACROSS THE GLOBE

Learning from what has worked and what has not in supporting vaccine developers during this pandemic is an ongoing mission for research infrastructures such as EATRIS. Taking knowledge from collaborators across the globe also highlights again the lessons not learned from previous pandemics, including, for example, issues in open and

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real-time sharing of precompetitive data. This remained a major roadblock to rapid and efficient vaccine and therapy development for COVID-19. Building flexible infrastructures to provide the right tools and expertise when needed and providing the funding at the right time also remained a major challenge in the present pandemic. This commentary piece has outlined some of the initiatives that aimed to overcome this – in particular, accessing the right preclinical tools – but more work is clearly needed.

Translation Together is a unique collaboration of leading translational research organizations from around the world including AdMare Bioinnovations (Canada), LifeArc (UK), NCATS (US), TIA (Australia), Fiocruz (Brazil), AMED (Japan), and EATRIS (Europe). In early 2022, the partners of Translation Together published an article in Nature Reviews Drug Discovery [10], reflecting on successes and challenges in regional COVID-19 pandemic responses and proposing five priorities to improve preparedness for future pandemics. In this publication, we draw on experiences and lessons learned in the COVID-19 pandemic to propose actions to improve the preparedness of the translational research community for future public health crises and to improve global health [10]. The take-home message remains clear: providing the right tools for efficient vaccine development programs needs the right infrastructures that can provide innovative, essential technologies and services in the most flexible manner. Achieving this goal represents a formidable opponent to any infectious disease pandemic we may face in the future. In particular, having a fast and effective system for vaccine development that can respond to the challenge of new, emerging variants in the present or future infectious disease pandemics. In addition, building flexible

FIGURE 2

ISIDORe consortium partners.

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funding schemes to promote access to these services such as the goal of ISIDORe, is also key to making accessible, emergency funds available to accommodate the global health priority of that time. With important lessons learned and new initiatives implemented, research infrastructures have clearly shown that they represent an essential player in accelerated vaccine development. A lot has been done, but there is much more to do.

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Personalized cancer vaccines: more is more?

We caught up with Geneos Therapeutics Founder Niranjan Sardesai to learn more about the company's highly personalized cancer vaccines, which target up to 40 tumor neoantigens in a single DNA plasmid vaccine.

Charlotte Barker, Editor, *Vaccine Insights* speaks with **Niranjan Sardesai,** Founder, President & CEO, Geneos Therapeutics.

NIRANJAN SARDESAI is Founder, President, and CEO of Geneos Therapeutics. He is recognized as an expert in nucleic acid vaccines and immunotherapies and led the development of the DNA medicines technologies that are at the core of Geneos' GT-EPIC™ platform. Previously he served as Chief Operating Officer and Head of R&D at Inovio Pharmaceuticals. He was responsible for the development pipeline and led the capital-raising efforts for the company via strategic out-licensing of pipeline products to secure major licensing deals totaling over $1 Bn and securing over $150M in non-dilutive grants and contracts from funding agencies. Dr. Sardesai received a PhD in Chemistry from the California Institute of Technology and an MBA from the Wharton School of the University of Pennsylvania, where he was the recipient of the Shils-Zeidman Award in Entrepreneurship

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Q What excites you about the field of cancer vaccines?

NS: I am excited by the notion that with cancer vaccines, you are training a person's own immune system to produce responses that can treat or fight disease. It is like the old saying that if you give a man a fish you can feed him for a day, but if you teach him how to fish you can feed him for a lifetime.

We know a lot about immunity and the components of immunity. However, there are still important unknowns. What are the rules for training the immune system, and more specifically, tailoring the immune system to drive the desired immune responses? How do we develop more potent and longer-lasting therapeutics and vaccines?

"As cancers progress, they start accumulating somatic changes that confer certain properties to each person's individual tumor. Therefore, a 'one-size-fits-all' approach will not work in every case."

The cancer vaccine world presents substantial challenges. An effective vaccine requires the coming together of the right antigens, administered using the optimal platform modality(ies), in the optimal vaccination regimen, driving the correct immune responses (T cells with a cytolytic phenotype) to then lead to tumor-killing and measurable, durable clinical effects. Additionally, you are dealing with the notion of self-versus-foreign, and the breaking of tolerance. But we know that nature gets it right in some patients with some antigens. These are the patients with the tumor-directed immune cells infiltrating the tumor (inflamed phenotype). These patients benefit from, for example, immune checkpoint inhibitor (ICI, PD1/PDL1) therapy.

Q What approach does Geneos Therapeutics take?

NS: Geneos Therapeutics is a clinical-stage immuno-oncology company focused on developing highly personalized immunotherapies for treating cancer, based on our proprietary GT-EPIC™ platform. We recognize that cancer is unique to an individual in many ways. Two individuals may have the same type of cancer in the same organ but at the genomic level, each person's cancer is very different. As cancers progress, they start accumulating somatic changes that confer certain properties to each person's individual tumor. Therefore, a 'one-size-fits-all' approach will not work in every case.

Geneos is targeting neoantigens. As cancer cells divide, their replication machinery is not perfect and changes in the cancer genome accumulate over time. Some of the changes are beneficial to the tumor in driving tumor growth and proliferation, including mutations in several well-characterized genes and pathways. But the changes are stochastic and can end up being the Achilles' heel of the tumor, as they flag cancer cells to the host immune system. We seek to train the patient's immune system to recognize these somatic changes (known as neoantigens) and target the cells for killing, using a DNA vaccine approach.

Our lead program is treating patients with advanced hepatocellular carcinomas (HCC), in whom multiple prior lines of therapy have failed. Traditional immunotherapeutic approaches, like ICIs, are not very effective in this setting, because liver cancers are largely immune-excluded (cold) tumors, are largely microsatellite stable tumors, and are largely low tumor mutational burden tumors. A key challenge in liver cancer is driving T cells into these tumors.

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Q How do you generate these personalized vaccines?

NS: We use a six-step process for developing GT-EPIC™ personalized therapies.

- Take a tumor biopsy sample and a normal sample from the patient.
- Isolate tumor DNA and mRNA and sequence the tumor sample through whole-exome and transcriptome sequencing.
- Evaluate the differences in the tumor DNA relative to the patient's own normal cells and identify targetable neoantigens.
- Design a patient specific optimized DNA insert that encodes for these neoantigens. This insert is then cloned into a DNA expression plasmid.
- Make the patient-specific product under GMP conditions. The product is tested for quality compliance and released for treating the patient.
- Treat the patient with their own personalized cancer vaccine.

Q How does this approach differ from others in development?

NS: The common misperception of individualized treatments is that they take too long to manufacture, cost too much, and are not as efficient as drugs that can treat multiple patients. We want to challenge that view.

There are three key differentiators for our platform relative to other similar approaches. The first is immune potency – the ability of the vaccine to drive the right kind of immune response in the right magnitude. One of the key requirements for a therapeutic vaccine is the ability to drive the optimal T-cell responses and we believe that how you deliver antigens is just as crucial as the antigens themselves to accomplish this. With our DNA-based approach, we can drive not only CD4+ T cells but also CD8+T cells against the targeted antigens.

The second is the number of antigens you can use to treat patients. There is a lot of ongoing work to develop algorithms to predict the best antigens to target; a goal that may be driven by the limitations in antigenic load carrying capacity or manufacturability of the other immunization approaches. We have taken the approach of treating the patient with all of their targetable antigens and then letting the patient's own immune system determine which of these antigens drive immune responses and lead to clinical efficacy. By going in with a larger repertoire of antigens, we can drive a broader/polyclonal T cell response to better engage and kill cancer cells using multiple potential MHC-antigenic peptide targets. We are also in a better position to deal with the polyclonal and multifocal nature of cancers, as well as metastatic disease. When it comes to targeting cancer with vaccines, our pre-clinical data and clinical results point to 'more is truly better!

The third aspect is turnaround time. We are dealing with advanced cancer patients, so time is critical. Today, we can go from biopsy to treatment in 6–8 weeks. By better integrating processes, we can bring that down to 3–4 weeks.

Q How much similarity is there in neoantigens between patients?

NS: This is a key question that the field is grappling with. What is clear is that most neoantigens identified in a patient tumor are specific to that patient. Mutations accumulate in tumors in an idiosyncratic manner, and the rate and the location of the mutations in the tumor genome vary extensively from patient to patient.

"In other pre-clinical studies modeling polyclonal or multifocal tumors, we have shown that the larger neoantigenic payloads are able to better protect the animals from a multiple tumor challenge."

There are a group of mutations or somatic changes that have been classified as driver mutations, which can be common across various patients, but these tend to be a small minority of the cumulative somatic changes that occur in a tumor. Some groups are focusing on these common mutations (i.e., shared neoantigens), that might be present to create "off-the-shelf" cancer vaccines with an eye to offsetting some of the manufacturing challenges associated with the other vaccine platforms.

With the advantage of reduced manufacturing complexity associated with DNA plasmids and a rapid manufacturing turnaround time, we are able to treat the patient with all their targetable antigens, regardless of whether they are shared or private; driver or passenger; branch or truncal. Our platform allows us to have larger antigenic payloads delivered to the patient and we are already treating patients with up to 40 neoantigens in clinical trials.

Q What results have you seen so far?

NS: In the preclinical setting, we have shown that we can deliver 60 neoantigens in mouse tumor models with no antigenic interference or competition [1]. Similarly, we have combined DNA plasmids encoding multiple full-length viral or tumor associated antigenic proteins that together represent several hundreds of potential antigenic sequences without impairing the immune responses or efficacy in animal challenge models. In other pre-clinical studies modeling polyclonal or multifocal tumors, we have shown that the larger neoantigenic payloads are able to better protect the animals from a multiple tumor challenge [2].

We are excited about the emerging results in a clinical setting. We are treating patients with advanced HCC with a personalized cancer vaccine in combination with a PD-1 checkpoint inhibitor, pembrolizumab in a second-line setting. HCC is the fourth most common cause of cancer-related death. Patients are typically diagnosed with advanced disease with poor 5-year survival rates and limited treatment options. ICIs targeting PD-1 have limited activity in HCC as monotherapy, with response rates ranging from 14–17%. We presented data at the Society for Immunotherapy for Cancer 2021 meeting from the first 12 patients treated in our study [3]. We showed that by including up to 40 epitopes in the vaccine we were able to target all neoantigens present in 83% of the patients. The objective response rate

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was 25% and the disease control rate was 66.7% (3/12 partial response, 5/12 stable disease, 4/12 progressive disease). We analyzed the TCR repertoire in peripheral blood and matched tumor tissue. We observed both novel and significantly expanded T cell clones post-vaccination in all patients analyzed. Many of the novel peripheral T cell clones were also identified to have trafficked to the tumor microenvironment post-vaccination, potentially mediating the observed tumor regression.

We have a second program in brain cancer, in the adjuvant setting. Here, we are treating patients with their personalized cancer vaccine alone, i.e., without combination with an ICI. Our first patient is now four years out from their primary surgery. The patient continues to remain on personalized cancer vaccine treatment, recurrence-free for over three years since initiating the adjuvant therapy [4]. We have performed extensive immune characterization to show that we are driving neoantigen-directed CD4+ and CD8+ T cells to the majority of the vaccine-encoded neoantigens and the T cells may account for the sustained effective tumor immune surveillance.

Q Do you use adjuvants?

NS: Our cancer vaccines are being delivered in combination with the immune cytokine IL-12. Two DNA plasmids are co-formulated and co-administered intradermally into the patient – one is a personalized DNA plasmid that encodes for cancer antigens, and the second is an IL-12 encoding plasmid.

The expression of IL-12 and antigens is co-localized to the injection site in the periphery (as opposed to the tumor microenvironment) and the IL-12 cytokine helps potentiate a Th1 immune response. Our mechanism of action is to drive CD8 T cells, which is augmented by having the IL-12 present locally at the injection site.

Q Why have so few cancer vaccines made it to market?

NS: It is difficult to speculate on the historical reasons, but the new generation of vaccine platforms developed for increased immune potency, better choice of antigens and adjuvants, and clinical trial settings are all pointing to an optimistic present for cancer vaccines. We are part of a new wave of cancer vaccine developers taking a more ground-up and methodical approach to vaccine development. Rather than basing early-stage clinical success solely on the detection of clinical response in small, single-arm uncontrolled studies, we are additionally carrying out extensive immune characterization to confirm that the observed clinical response is mechanistically supported by detectable immune responses in the patients. Are we driving T cell responses with a CTL phenotype and are the activated T cells infiltrating the tumor?

Cancer vaccines have demonstrated a strong safety profile during development and could make attractive combination partners with ICI. Cancer vaccine plus ICI combinations have the potential to increase ICI efficacy without adding to the ICI safety/toxicity profile which has often been the case with other cancer drug–ICI combinations or ICI–ICI combinations. In addition to the advanced disease settings, cancer vaccines are ideally positioned to play an

effective role in earlier disease settings and for treating chronic diseases – settings where the safety/toxicity profile is not favorable for ICI immunotherapy or other forms of cytotoxic therapies.

I am upbeat about cancer vaccines. The emerging data is exciting. We are seeing cancer vaccine driven tumor reductions and objective clinical responses in our clinical studies. All the science from us and our peers in the field is pointing toward cancer vaccines being transformative and taking their rightful place in the therapeutic regimens available to patients.

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AUTHORSHIP & CONFLICT OF INTEREST

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