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Conference report

Mind the gap from research laboratory to clinic: Challenges and opportunities for next-generation assays in human diseases

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ABSTRACT

Modern vaccinology has experienced major conceptual and technological advances over the past 30 years. These include atomic-level structures driving immunogen design, new vaccine delivery methods, powerful adjuvants, and novel animal models. In addition, utilizing advanced assays to learn how the immune system senses a pathogen and orchestrates protective immunity has been critical in the design of effective vaccines and therapeutics. The National Institute of Allergy and Infectious Diseases of the National Institutes of Health convened a workshop in September 2020 focused on next generation assays for vaccine development (Table 1). The workshop focused on four critical pathogens: severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) and human immunodeficiency virus (HIV)—which have no licensed vaccines—and tuberculosis (TB) and influenza—both of which are in critical need of improved vaccines. The goal was to share progress and lessons learned, and to identify any commonalities that can be leveraged to design vaccines and therapeutics.

1. Current landscape of clinical trials and correlates of protection

The first session of the workshop focused on clinical trials infrastructure and use of assays to identify correlates of protection in the context of trials. As the coronavirus disease 2019 (COVID-19) pandemic emerged in the United States and around the world, programs and investigators from the HIV field and other areas of immunology/virology have adapted their expertise and technologies to quickly investigate vaccines, therapeutics, and diagnostics for SARS-CoV-2. NIH brought together the resources and expertise of four existing NIH networks—the HIV Vaccine Trials Network (HVTN), Infectious Diseases Clinical Research Consortium (IDCRC), AIDS Clinical Trials Group (ACTG), and HIV Prevention Trials Network (HPTN)—to create the COVID-19 Prevention Network (CoVPN) to develop and conduct vaccine efficacy and mAb prevention trials (presented by Jim Kublin) [1,2]. The CoVPN optimized site selection and enrollment through collaboration with a predictive analytics effort—the HHS Protect Dashboard—that was launched to provide pathogen- and society-associated data and guidance to CoVPN and other federal COVID-19 efforts, including predictive modeling and early infection data (presented by Julie Ledgerwood) [3]. This approach has enabled the CoVPN to expeditiously activate more than 150 clinical trial sites in areas across the United States and internationally in epidemiological "hot spots."

CoVPN is working with multiple vaccine developers using a variety of vaccine designs, including mRNA, plasmid, and recombinant protein approaches. Among the companies partnering with CoVPN is Janssen, which shared their early-stage results at the workshop. Janssen repurposed its adenovirus 26 platform—currently being tested for vaccines against Ebola, HIV, respiratory syncytial virus, and Zika—to include the gene for the SARS-CoV-2 spike protein (presented by Maria Grazia Pau) [4]. Ad26-based vaccine

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Table 1
Meeting Steering Committee and Presenters. Names in bold font in text are Presenters whose work is cited.

Sarah Andrews, National Institute of Allergy and Infectious Diseases, Bethesda, MD
Kristen Cohen, Fred Hutchinson Cancer Research Center, Seattle, WA
Shane Crotty, La Jolla Institute for Immunology, La Jolla, CA
Anna Durbin, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Genoveffa Franchini, National Cancer Institute, Bethesda, MD
Michael Gale, University of Washington, Seattle, WA
Ivelin Georgiev, Vanderbilt University Medical Center, Nashville, TN
Peter Gilbert, Fred Hutchinson Cancer Research Center, Seattle, WA
Hana Golding, U.S. Food and Drug Administration, Silver Spring, MD
Raphael Gottardo, Fred Hutchinson Cancer Research Center, Seattle, WA
Maria Grazia Pau, Janssen Vaccines & Prevention B.V., Leiden, NL-ZH
Walid Heneine, Centers for Disease Control and Prevention, Atlanta, GA
Purvesh Khatri, Stanford University, Stanford, CA
Steven Kleinstein, Yale School of Medicine, New Haven, CT
Florian Krammer, Icahn School of Medicine at Mount Sinai, New York, NY
Jim Kublin, Fred Huchinson Cancer Research Center, Seattle, WA
Julie Ledgerwood, National Institute of Allergy and Infectious Diseases, Bethesda, MD
Robin Levis, U.S. Food and Drug Administration, Silver Spring, MD
John Mascola, National Institute of Allergy and Infectious Diseases, Bethesda, MD
David O'Connor, University of Wisconsin - Madison, Madison, WI
Gabriel Ozorowski, The Scripps Research Institute, La Jolla, CA
Isabela Pedroza-Pacheco, University of Oxford, Oxford, OXF
Bali Pulendran, Stanford University, Palo Alto, CA
Thomas Scriba, University of Cape Town, Cape Town,
Alessandro Sette, La Jolla Institute for Immunology, La Jolla, CA
Alex Shalek, Massachusetts Institute of Technology, Cambridge, MA
George Shaw, University of Pennsylvania, Philadelphia, PA
Georgia Tomaras, Duke University, Durham, NC
John Tsang, National Institute of Allergy and Infectious Diseases, Bethesda, MD

vectors have been shown to induce a polyfunctional antibody response and CD4/8 T cell responses with a Th1 signature. The US FDA granted Emergency Use Authorization to Janssen's SARS-CoV-2 vaccine in February 2021.

Once additional understanding of SARS-CoV-2 immunology, and specifically vaccine immune responses that predict protection against COVID-19 are acquired, it might be possible to accelerate approval and licensure of a COVID-19 vaccine based on a surrogate endpoint (e.g., immune response) that is reasonably likely to predict clinical benefit. The CoVPN trials are using a harmonized vaccine protocol developed by the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) public–private partnership that will enable analyses of correlates of protection across multiple trials [1]. Many assays being used to measure immunological endpoints for CoVPN trials have been developed or refined by NIAID-funded investigators for HIV vaccines; these include spike protein binding antibody, neutralizing Ab (nAb) assays using live virus or pseudovirus, and T cell assays. Florian Krammer highlighted the development of an ELISA to measure serological response to SARS-CoV-2, similar to assays developed for influenza. The assay is being used to assess the correlation between serologic response and nAb in SARS-CoV-2-infected patients and determine whether a protective titer against SARS-CoV-2 can be established [5,6]. Mechanistic studies are also being used to support the case for immunologic biomarkers. The CoVPN Statistical Center is working with laboratory researchers to identify biomarkers—including binding Ab and nAb biomarkers—with broad dynamic range and limited technical measurement error with the goal of establishing correlates of protection (presented by Peter Gilbert) [7].

Having a common assay to evaluate correlates of protection allows for comparability of data across studies. For example, a clinical assay for Ebola virus vaccines (glycoprotein ELISA) was developed collaboratively through the Filovirus Animal Nonclinical Group (FANG). A single assay was validated for nonhuman primates (NHP) and human samples, which was important because Ebola vaccines may be approved under the Animal Rule. The assay was reviewed under a single Master File, which can be cross-referenced by any vaccine developer. There are similar efforts in the United States and globally to coordinate assay development for SARS-CoV-2. Moreover, if there is demonstration of protection in one population, effectiveness of the same vaccine could be inferred in other populations, such as pediatrics, by comparing immune response biomarker(s) between populations using pre-specified criteria. Provided assays are well qualified, regulators are willing to be flexible with respect to assay development and use, particularly for use with nonclinical data studies and assessments of risk-based approaches in testing and trial design (presented by Robin Levis).

These SARS-CoV-2 studies are taking advantage of decades of past research experience in other infectious diseases. Mechanisms—and correlates—of protection vary across pathogens. For example, the HIV broadly neutralizing antibody, VRC01, targets the conserved CD4 binding site of the HIV envelope [8,9] and has been demonstrated to prevent HIV infection in non-human primate (NHP) challenge studies [10]. Building on these NHP results, the first efficacy studies of VRC01—termed the Antibody Mediated Prevention (AMP) trials [11,12]—for HIV prevention were launched to determine whether a serum titer of nAb can be established as a biomarker of protection in humans (presented by John Mascola). The internationally conducted AMP trials demonstrated protection from acquisition of HIV isolates sensitive to neutralization by VRC01, providing a proof-of-concept for prophylaxis with broadly neutralizing antibodies [13]. Future trials with improved antibodies and cocktails are planned. In addition to potentially leading to a new HIV prevention modality, evaluating the preventive efficacy of mAbs will inform future HIV immunogen design by providing an assessment of the amount of neutralizing antibody required to achieve protection [14]. In the TB field, researchers are exploring correlates of protection afforded by BCG, the only approved TB vaccine, as well as a novel polyprotein-adjuvant TB vaccine candidate. Protection induced by the BCG TB vaccine against TB disease in adolescents and adults is highly variable, and there are currently no known correlates of protection. However, recent NHP studies suggest that antigen-specific Th1 and Th17 responses and IgA antibodies in the lungs correlate with BCG-induced protection against TB [15,16]. These measures will be explored in trials testing the efficacy of BCG revaccination and a polyprotein-adjuvant TB vaccine (presented by Tom Scriba).

2. Next generation tools and technologies

Advanced technologies allow for interrogation of how the immune system responds to a vaccine, or to infection, and of the epitopes on an immunogen that elicit immune responses. To develop enhanced immunogens, researchers can use paired heavy and light chain B cell sequencing analysis to better understand clonal expansion and somatic hypermutation (SHM) for infectious agents, such as HIV (presented by Kristen Cohen) [17] and influenza (presented by Sarah Andrews) [18]. By using multiplexed fluorophore-tagged proteins, lymphocyte antigen specificity is linked to single-cell receptor sequencing. These data are applied to develop immunogens that can drive SHM and development of broadly neutralizing antibodies (bnAbs) (presented by Kristen Cohen) and to identify cross-reactive B cell receptors responding to influenza vaccination (presented by Sarah Andrews). LIBRA-Seq expands upon the use of fluorophore-tagged proteins to include oligo-tagged proteins. In principle, this technique can be

very highly multiplexed to accommodate and resolve unlimited epitopes. Using LIBRA-Seq, researchers can build high-resolution antigen specificity maps (presented by Ivelin Georgiev) [19]. Similarly, the structural techniques electron microscopy-based polyclonal epitope mapping (EMPEM) [20] and cryogenic electron microscopy (CryoEM) [21] permit very detailed mapping of epitope binding from polyclonal sera. These techniques can be applied rapidly and accommodate native antigens, providing significant advantages over traditional crystallography (presented by Gabe Ozorowski). The advancement of these approaches is enabling researchers to elucidate the structural basis of virus-antibody interactions and improve immunogens for a variety of infectious agents. However, the inability to predict lymphocyte antigen specificity from antigen receptor sequences remains a major gap.

Interrogation of the host/recipient response is another critical aspect of vaccine design. Development of activation-induced marker (AIM) assays (presented by Shane Crotty) has overcome two major technical limitations: identification of germinal center T follicular helper cells (GC Tfh), which do not produce high levels of cytokines, and identification of live, antigen-specific T cells, in contrast to cytokine detection methods that require fixation [14,22–24]. This technique has allowed more sensitive detection of T cells responding to a given antigen and can be applied to study responses in lymphoid tissue, complementing existing tools for detecting cellular immune responses that includes flow cytometry-based approaches (tetramers, intracellular cytokine staining, cytometry by time of flight (CyTOF)), and tissue culture-based assays (cytotoxic target killing assays, ELISpot). In parallel, application of lymph node fine needle aspirates (FNAs) has enhanced the ability to interrogate adaptive immune responses at the anatomical sites where they occur (presented by Shane Crotty and Kristen Cohen) [25]. Recently developed techniques for single-cell analysis allow characterization of cellular heterogeneity at unprecedented resolution, as well as the unmasking of rare cell populations. Of particular relevance, advances in single-cell RNA sequencing technology, such as the Seq-Well platform, have dramatically increased utility and access by lowering cost and equipment barriers (presented by Alex Shalek) [26]. These technologies can be applied to specimens obtained in the context of human (or model system) trials to examine immune homeostasis and uncover correlates (or mechanisms) of successful or failed responses. Full sample processing on experimental medicine or subsets of samples may make the most sense however due to cost and complexity, with careful banking of samples to enable subsequent processing guided by clinical observations.

The technologies discussed above are most productively applied to early-stage clinical or pre-clinical development in order to understand mechanism and drive development of better vaccines. These rapidly evolving technologies are highly specialized, protocol specific assays to support iterative product development and not easily amenable to industry-level standards for assay validation. These approaches are better suited to exploratory endpoints, rather than primary validated or qualified assays that enable reliable quantitative assessments of immune functions and specificities across different vaccine platforms (Fig. 1).

3. Integrating data to define immune signatures of infection and vaccination responses in humans

Integration of multidimensional data using systems immunology approaches can generate signatures that characterize how, where, and when hosts fight specific pathogens or respond to vaccination, informing therapeutic and preventive strategies. As one example, researchers are integrating multi-omics data to predict systems level disease severity in COVID-19 patients. Data from mass cytometry, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), and *ex vivo* stimulation of plasmacytoid dendritic cells collectively suggest spatial and temporal dichotomies in the immune response in COVID-19. Specifically, research has found excessive proinflammatory responses in the lung, and suppressed innate immunity in the peripheral blood. Additionally, there is a low-level, transient spike in IFN-α early in disease that leads to later upregulation of IFN-stimulated genes. Differences between moderate and severe disease have been observed (presented by Bali Pulendran) [27].

Leveraging biological, clinical, and technical heterogeneity across multiple diverse datasets has been repeatedly shown to identify generalizable signatures in tuberculosis [28] and sepsis [29]. This approach was used to identify a conserved host response to viral infections: a *meta*-virus signature (MVS) [29]. Its association with disease severity across multiple viruses was demonstrated using greater than 5000 samples from patients with one of 16 viral infections from 18 countries [30]. The MVS was validated using bulk RNA-seq data from 62 SARS-CoV-2 patients from Greece, and scRNA-seq of 702,000 immune cells from 289 samples across three clinical centers in the US. Further analysis showed that the MVS score is higher in myeloid cells, particularly CD14 monocytes. The number of CD14 cells increased with severity of disease, and the transcriptional profiles of these cells change. This work has led to the identification of a 5-gene signature for predicting severity of SARS-CoV-2 infection, which will be developed into a qRT-LAMP-based assay for point-of-care test (presented by Purvesh Khatri) [31].

Systems immunology also can be used to characterize responses to infection and vaccination and inform iterative improvements in vaccine design. In the field of HIV research, data from high-dimensional assays for viral and host factors, global antibody repertoire, cellular immune environment, and transcription were integrated to define signatures of the immunological environment permissive to generation of HIV-1 bnAbs (presented by Isabela Pedroza-Pacheco). Correlation network analysis suggested that hyper-immune activation and activation of CD4 T cells plays important roles. These results suggest that vaccines should be designed to elicit high-magnitude, functionally potent CD4 Tfh responses that are targeted to provide help to bnAb-producing B cell lineages while limiting activation of regulatory cells.

Systems immunology is also accelerating adjuvant design. CITE-seq has been used to look at the molecular and cellular underpinnings of a previously identified 10-gene signature associated with influenza and yellow fever vaccine responses (presented by John Tsang). Results suggest that high responders have more activated plasmacytoid dendritic cells, elevated type I IFN signaling, and more persistently activated T and B cells at baseline [32]. This "naturally adjuvanted state" enables a stronger plasmablast response to vaccination or other triggers. Increased understanding of the natural adjuvanted state may guide development of engineered adjuvants.

Systems immunology can be facilitated by centralized data repositories and analysis tools. ImmuneSpace (https://www.immunespace.org) is an example of a data management and analysis engine that was created to facilitate research on data from the Human Immunology Project Consortium (HIPC) using cutting-edge computational tools. The goal of this resource is to characterize the status of the immune system in diverse populations under both normal conditions and in response to stimuli (presented by Raphael Gottardo) [33]. The Immune Epitope Database (IEDB http://www.iedb.org/) is another systems tool that houses experimentally derived epitope information from the published literature and direct submissions, primarily NIAID Epitope Discovery contracts. IEDB features a sophisticated search interface, as well

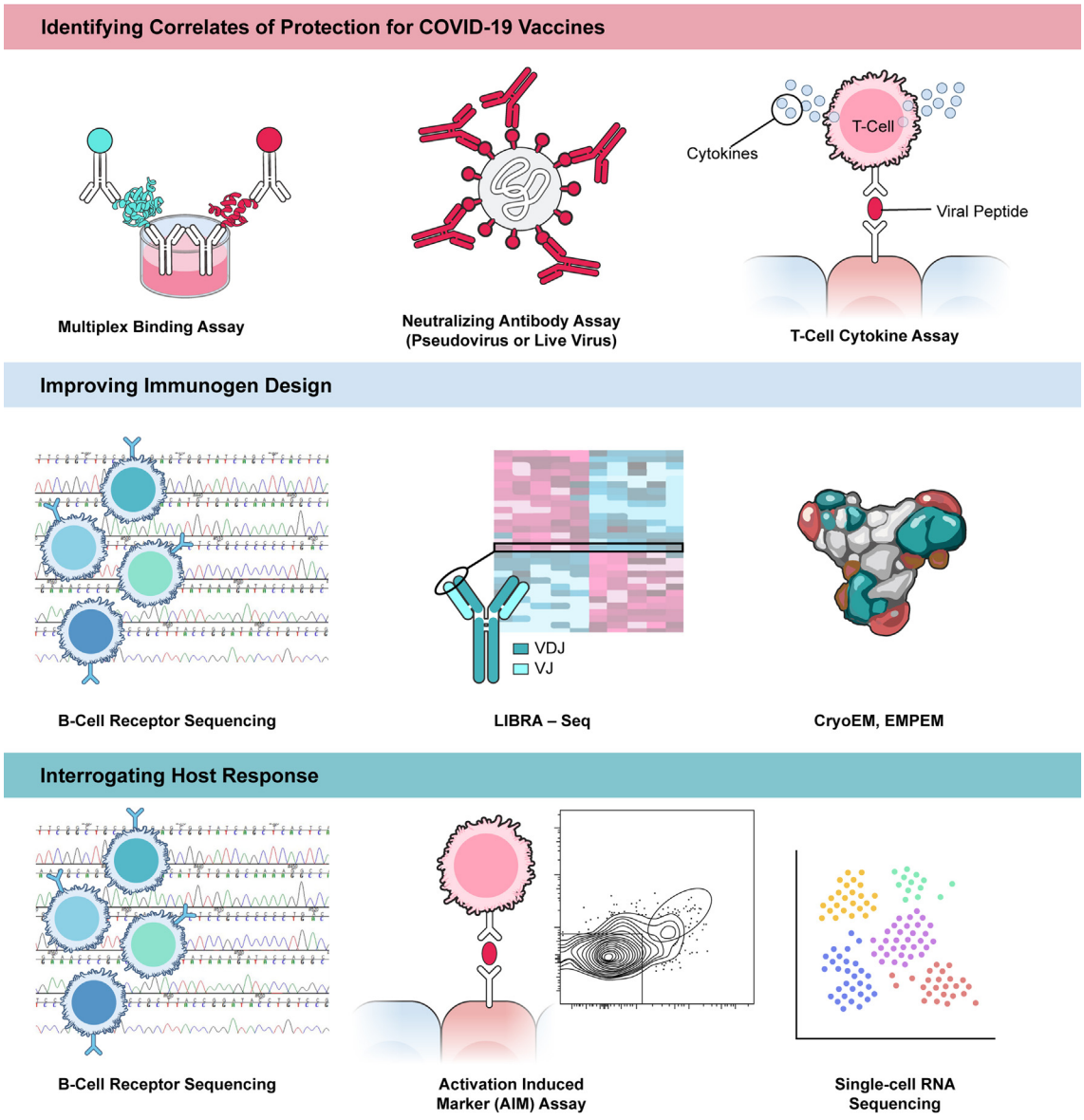

Fig. 1. Next generation assays highlighted at this workshop. This illustration represents a selection of the next generation assays presented in the workshop and their applications by researchers to study correlates of protection, improve vaccine design, and investgate the host immune response.

as several visualization and analysis tools (presented by Alessandro Sette) [34].

4. Developing and translating immune and protection correlates in Non-Human primate models

The last session covered the use of NHP models to conduct immune correlate studies that are difficult or impossible to do in humans, such as investigating immunological features associated with protection imparted by SIV vaccines. A study of whole-blood transcriptome profiling of rhesus macaques that received a 68–1 RhCMV/SIV vaccine identified a protective gene expression signature involving IL-15. This signature was significantly higher in nonprotected animals at baseline. Vaccinated macaques that were protected from SIV challenge tended to have a more quiescent IL-15 signaling pathway at baseline and manifested post-vaccination enhancement of IL-15 signaling (presented by Michael Gale). Maintenance of the protective profile was essential for protection from disease. Differences were driven mainly by monocytes, with contributions from T cells and NK cells. These data suggest that IL-15 response could be a pivotal factor in programming RhCMV/SIV vaccine-induced immune protection [35].

In another SIV study, a variety of assays—including conventional immunoassays and assays measuring transcriptomes, extracellular vesicle mRNAs, and the epigenome—were used to assess correlates of risk and protection in the SIV_{mac251} model (presented by Genoveffa Franchini; Bissa et al. in preparation) [36–39]. Collectively, these data suggest roles for anti-inflammatory macrophages, NK cells, Th1 cells, antibodies to V2, and Treg cells in vaccine-mediated protection from mucosal infection. Results from multiple assays pointed to differences in regulation of CREB signaling being associated with protection. This is consistent with human data from RV144 showing a trend towards higher levels of CREB1-driven genes in protected patients (Sekaly, R under revision, *Nature Immunology*).

To explore bnAb development, researchers used clinically relevant simian-human immunodeficiency virus (SHIV) challenge stocks to study bnAb generation in rhesus macaques (presented by George Shaw) [14]. The bnAbs elicited in macaques target mul-

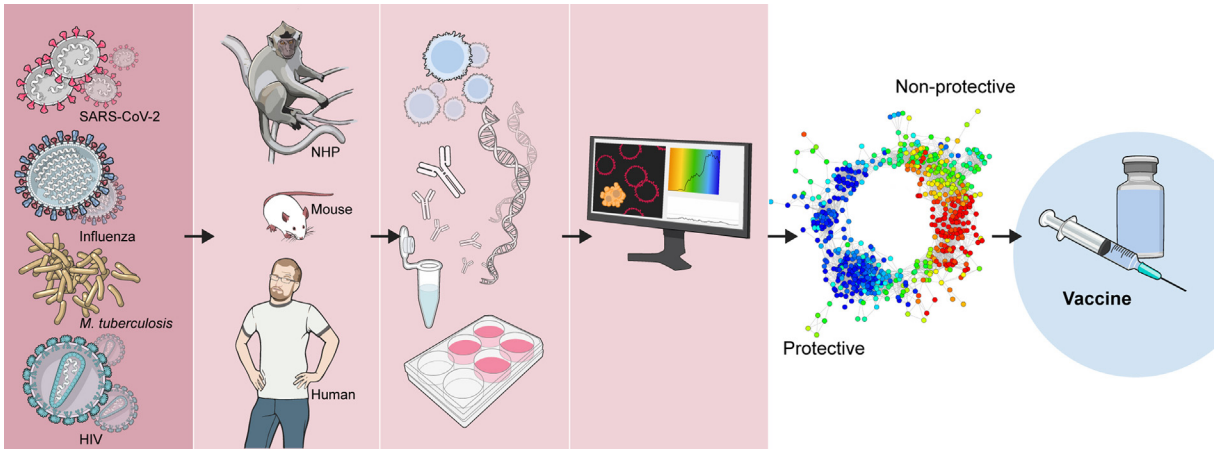

Fig. 2. Integration across pathogens, species, and assays to drive development of improved vaccines. Data from humans, non-human primates, and small animals infected with, or vaccinated for, influenza, HIV, SARS-CoV-2, or *M. tuberculosis* can be integrated, cross-validated, and interrogated with advanced computational tools. A wide variety of established and novel assays can be applied to uncover the responses to infection and vaccination with these and other pathogens.

Table 2
Potential Recommendations for Assay and Reagent Standardization.

Considerations for Use of Laboratory Assay Data	
Scenario	**Recommendation**
Generate reliable data to inform regulatory strategies about vaccine critical path	• Validated Assays • GCLP • Common Reagents
Compare various vaccine regiments	• Validated Assays • GCLP • Common Reagents
Define correlates, surrogates, and mechanisms of vaccine-induced protection, duration of protection	• Archived Specimens • Multi Disciplinary Expertise • Advanced Planning
Build infrastructure to conduct studies across different pathogens from participating sites globally	• Invest in Local Staff Training • Abide by In-country Regulations • Knowledge of Circulating Variants

tiple epitopes and closely resemble HIV-1 bnAbs. The presence of bnAbs *in vivo* elicited escape through mutation of residues 166 and 169, suggesting these residues are sufficient for driving affinity maturation leading to bnAb breadth and potency. These findings validate the rhesus model for HIV-1 vaccine research. In particular, analysis of Env-Ab coevolution leading to neutralization breadth in SHIV-infected macaques could help guide HIV-1 vaccine design and testing [40].

NHPs also have been used to evaluate attenuated dengue strains for vaccination and use in controlled human infection models (CHIMs; presented by Anna Durbin) [41]. Based on NHP data, two strains developed from the DENV-2 Tonga/74 virus—DEN2Δ30 and DEN3Δ30—were evaluated as challenge strains. In a human challenge study, all human subjects developed viremia and the vast majority developed dengue rash when exposed to the challenge strains, but none developed signs of serious infection. Two candidate dengue vaccines—TV003 and TV005—were found to completely protect against viremia induced by the DENV2 challenge strain in early-phase trials [42]. TV003 currently is being evaluated in a phase 3 trial. Efficacy results should be available in about one year.

These examples illustrate the value of using NHPs to explore vaccine-induced protective (and non-protective) signatures or biomarkers in advancing product development without costly human trials. Systems analyses in NHP models are able to interrogate the timing of these signatures, and to allow collection of invasive tissue specimens which are typically not possible in human studies.

5. Key themes and next steps

Several key themes emerged from workshop presentations and discussion. The rapid planning and launch of SARS-CoV-2 clinical trials and the surge of knowledge about SARS-CoV-2 and COVID-19 illustrate the value of investment in clinical and research infrastructure for infectious disease research at a global level. Following direct demonstration of protection in one population, effectiveness of the same vaccine could be inferred in other hard to recruit pop-

ulations by immunobridging, using validated immunological assays and by comparison of immune response biomarker(s) between populations using pre-specified criteria. These immune assays can serve to predict clinical benefit as well as effectiveness as immunity wanes over time. Merging cutting-edge technologies provides unprecedented opportunity to learn more about immune responses at the systems level (Fig. 2). However, this work can present technical challenges, as the high-throughput data requires extensive computational mining to generate novel hypotheses that can guide future investigational product design. These omics-based technologies often require expensive equipment and technical knowledge that are not readily transferrable between research groups. Because of its complexity, this work will require targeted funding and fostering of collaborations to promote optimal application of these technologies across pathogens. Investments in technological improvements are needed to move toward validation of these assays and tools and large-scale use, including in the context of clinical trials. Existing data should be leveraged to the extent possible, including use of centralized data repositories and analysis resources. New studies should be thoughtfully designed to ensure that informative samples are available to address a range of high-value research questions now and in the future (Table 2). And to address the substantial analytical challenges associated with large, multimodal datasets, efforts should be made to increase bioinformatics expertise within the immunology and vaccine development fields. Investments in these areas, and the support of a diverse biomedical workforce, will create the learning environment required to conduct effective and cutting-edge translational research globally and ensure a pipeline of highly-skilled investigators poised to advance the next generation of vaccines and therapeutics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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