Workshop Report

COVAX Enabling Sciences SWAT Team Workshop on *"Global and Local Efforts to Detect and Interpret SARS-CoV-2 Variants"*

April 16, 2021

Meeting report prepared by

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Executive summary

On 16 April 2021, the COVAX Enabling Sciences SWAT Team hosted a workshop on "*Global and Local Efforts to Detect and Interpret SARS-CoV-2 Variants*." The overall objective of this workshop was to share and connect information on the various facets of investigation such as pathogen genomic sequencing, epidemiology, virology, and immunology as efficiently as possible, and to enable rapid sharing of actionable information regarding the immunological consequences of the SARS-CoV-2 variants of concern (VoC) constellation.

The first half of the workshop featured presentations focused on describing the current local efforts to identify and interpret the impact of SARS-CoV-2 variants in four important global regions, South Africa (SA), Brazil (BR), the United Kingdom (UK) and India (IN). Key points included:

A. A recurring theme that expressed the crucial need for cross-disciplinary cooperation and willingness to share data and ideas both freely and completely. This was the single most important contributor to the successes within each of the regions represented in the discussion.

B. Access to and speed of genomic sequencing was identified as the cornerstone of variant detection; coupling to rapid immunological characterization of each variant was also emphasized as a strong contributor to success.

C. The sharing of such sequence data has made clear the global dispersed presence of many VoCs. Therefore, attribution to and nomenclature based on locales may cause unnecessary bias. There is a recognized need to end naming VoCs by country of first detection and adopting a more scientific convention (such a system has recently been introduced for discussion by the Global Initiative on Sharing Avian Influenza Data [GISAID]).

D. Re-purposing and building on assays developed for other viruses (e.g., HIV) has contributed to the speed with which meaningful data have been enabled.

E. Heterogeneity among vaccine-induced and convalescent immune repertoires exist. Immune escape remains a challenge. Monitoring difficulty is compounded by the heterogeneity in methods utilized to assess neutralization; harmonization and standardization is sorely needed to extract as much actionable information as possible from the wealth and diversity of data.

The second half of the workshop focused on global efforts and programs aimed at collecting and coordinating actionable data and information with the goals of standardizing approaches, facilitating faster and more effective responses, and enabling greater proactivity going forward. Key points included:

A. Rapidly developed vaccines may not be as effective against VoCs and global preparedness efforts must be redoubled going forward.

B. The WHO International Standard (IS) offers a simple mechanism for data sets to be compared between different organisations within each variant assay. Consistent with the COVAX Clinical Development & Operations and Enabling Sciences Joint Workshop on Immune Correlates held in February, there was strong support for its utility in "*harmonising the assessment of immune responses to COVID-19 vaccine and assessing the impact of variants*."

C. National and international coalitions such as COG-UK, the CEPI Agility Program, the US Government (USG) SARS-CoV-2 Interagency group (SIG), and NIH ACTIV / TRACE, and programs being introduced by the Wellcome Trust are foundational to establishing a working network that gathers, catalogs and makes available data and resources as well as informed analyses. Such efforts have yielded important insights such as the fact that new variants of SARS-CoV-2 appear to demonstrate varying levels of resistance to neutralization but cross-protective humoral immunity in convalescent sera against contemporary clades is still measurable.

D. Vigilance must be maintained with respect to quality of materials and data – especially regarding standardized and shared materials such as cell lines, viral stocks and reference standards.

E. Continued global cooperation and sharing is strongly encouraged and supported by members of the panel.

The final set of presentation materials from the workshop have been posted on the COVAX Epi Hub and can be found here: ***Global and local approaches to detect and interpret SARS-CoV-2 variants*****.**

This workshop, entitled *Global and Local Efforts to Detect and Interpret SARS-CoV-2 Variants*, was convened by the COVAX Enabling Sciences SWAT team. This team is co-led by the Coalition for Epidemic Preparedness Innovations (CEPI) and the World Health Organization (WHO) and includes members from the Bill & Melinda Gates Foundation (BMGF), the National Institute of Allergy and Infectious Diseases (NIAID) and private industry. COVAX is the vaccines pillar of the Access to COVID-19 Tools (ACT) Accelerator, co-led by Gavi, The Vaccine Alliance (Gavi), CEPI and WHO. COVAX supports the acceleration of the development and manufacture of COVID-19 vaccines, and, with special attention to Low and Middle Income Countries (LIMCs), aims to guarantee fair and equitable access to appropriate, safe and efficacious vaccines for all countries.

Agenda

Time (CET)	April 16, 2021	Speaker(s)
15:00 – 15:05	**Welcome and meeting objectives**	**Ivana Knezevic,** co-lead of ES SWAT team, WHO
Part I: Assessing emerging SARS-CoV-2 variants locally to inform the global response		
15:05-15:20	Variants of concern and interest in Africa: Scientific characterization in real time	**Tulio DeOliveira**, KRISP
15:20-15:30	Assessing immunological implications of VoCs in South Africa	**Penny Moore**, NICD
15:30-15:40	Immune escape and evidence for re-infection with P.1 in Brazil	**Ester Sabino**, Univ. Sao Paulo
15:40-15:50	Impact of B.1.1.7 on vaccine induced immune responses	**Ravi Gupta**, Univ. Cambridge
15:50-16:00	Understanding SARS-CoV-2 variants in 2021: Indian Perspective	**Anurag Agrawal**, CSIR/IGIB

16:00-16:25	**Panel Discussion**	Moderated by: **Karen Makar**, BMGF
16:25-16:30	**BREAK**	ALL
Part II: Contribution of international collaborations and consortia to the global effort		
16:30-16:40	WHO framework for response to new COVID vaccines	**Sylvie Briand**, WHO
16:40-16:50	Pulling it all together in the COG-UK	**Sharon Peacock**, COG-UK
16:50-17:00	CEPI's Agility program: a centralised approach to evaluate VoCs	**Simon Funnell**, PHE
17:00-17:10	Production of SARS-CoV-2 stocks	**Sujatha Rashid**, BEI
17:00-17:10	SARS-CoV-2 Interagency Group (SIG) Variant Assessment/Characterization	**Steve Oberste**, CDC
17:00-17:10	ACTIV/TRACE OpenData portal	**Christine Colvis**, NIH/NCATS
17:00-17:10	**Panel Discussion**	Moderated by: **Bill Dowling**, CEPI
17:00-17:10	**Wrap Up & Next Steps**	**Ivana Knezevic**

Welcome and meeting objectives

Dr Ivana Knezevic, WHO Team Leader of the Norms and Standards for Biologicals Group, and co-lead of the Enabling Sciences (ES) SWAT team (together with Paul Kristiansen, CEPI Head of Standards and Assay, Preclinical and Immunology) welcomed participants, thanked the workshop organizers, Karen Makar, Senior Program Officer at BMGF and William (Bill) Dowling, Non-Clinical Vaccine Development Leader at CEPI, and set the context for the workshop as described:

As data accumulates regarding known SARS-CoV-2 variants, and discoveries of new and sometimes novel variants are made, the importance of this workshop entitled *Global and Local Efforts to Detect and Interpret SARS-CoV-2 Variants* becomes clear. There have been and are planned many other events dedicated to understanding and managing the challenges presented by SARS-CoV-2 Variants of Concern (VoCs) that are so designated due to increased transmissibility or resistance to therapeutic intervention and/or vaccines.

The overall objective of the workshop is, therefore, to connect information on the various facets of investigation such as pathogen genomic sequencing, epidemiology, virology, and immunology as efficiently as possible and to enable rapid sharing of actionable information regarding the immunological consequences of the SARS-CoV-2 VoC constellation.

Specific topics and questions addressed in Part I of the workshop entitled, *Assessing emerging SARS-CoV-2 variants locally to inform the global response* included:

- What have we learned about immune escape from communities where variants have been detected?

- How can we enable more countries to rapidly generate interpretable data on immune escape that can inform local and global decisions, especially related to vaccine design and usage?

Specific topics and questions addressed in Part II of the workshop entitled, *Contribution of international collaborations and consortia to the global effort* included:

- Coordinating a global response and building consensus
- What opportunities are there for scientists to engage in international efforts?
- What resources are available to the international scientific community (reagents, standards, protocols, assays, data)?

Part I: Assessing emerging SARS-CoV-2 variants locally to inform the global response

Session 1: Variants of concern and interest in Africa: scientific characterization in real time

This session was presented by Prof. Tulio De Oliveira, PhD, Director of KRISP (KwaZulu-Natal Research and Innovation Sequencing Platform), and focused attention on current and planned efforts in South Africa and across the continent to leverage experience and knowledge gained over decades of research on HIV and drug resistance in studies of SARS-CoV-2. Emphasizing the established cooperation among the many organizations and individuals that comprise the Network for Genomic Surveillance South Africa (NGS-SA) and the Africa CDC Pathogen Genomics Initiative (PGI), Prof. DeOliveira acknowledged colleagues Prof. Koleka Mlisana and Prof. Alex Sigal for assisting in preparing the presentation.

Topics included:

- Description and update of NGS-SA and variants SA
- Effect of 501Y.V2 (B.1.351) in neutralization of first wave virus and vaccines
- Expanding genomic surveillance and genotype-to-phenotype to all of Africa

Summary:

1) The Network for Genomic Surveillance in South Africa (NGS-SA) was formed in May 2020 and includes 5 centers with expertise in genomics supported by grants from the South African Medical Research Council and the South African Department of Science and Innovation. https://doi.org/10.1016/S2666-5247(20)30116-6
2) A random sampling approach from over 300 different clinical facilities allowed sequencing of relatively few total samples and demonstrated the rapid displacement of many lineages present in SA by the 501Y.V2 (B.1.351) variant over the 6 months spanning Oct 2020 (11% of sequences) to Mar 2021 (97% of sequences); the approach also allowed the detection of the B.1.1.7 variant in the Western Cape, and A.23.1, a new variant of interest found originally in Uganda, in the eastern Cape. https://doi.org/10.1038/s41591-021-01255-3

3) The 501Y.V2 variant is characterized by 3 substitutions in the Receptor Binding Domain (RBD) (K417N, E484K and N501Y), and substitutions and a deletion in the N-terminal domain (NTD). https://doi.org/10.1038/s41586-021-03402-9
4) Widespread presence of the 501Y.V2 variant has been reported in 68 countries and naming variants by presumed country of origin may be inappropriate. https://doi.org/10.1126/science.abh0836
5) IMMUNE ESCAPE (Convalescent Plasma): Live virus neutralization assays conducted with convalescent plasma of first and second wave infected adults showed poor neutralization of the 501Y.V2 variant by first wave plasma (reduced 15.1-fold compared to neutralization by second wave plasma). However, second wave plasma was only 2.3-fold less effective against a first wave variant. Convalescent plasma of a person infected by a variant containing only the E484K substitution strongly neutralized both variants. https://doi.org/10.1038/s41586-021-03471-w
6) IMMUNE ESCAPE (Vaccines): Pseudovirus and Live virus assays correlate with respect to reduced neutralization of 501Y.V2 following 2 dose regimen of ChAdOx1 vaccine, 21% and 42% neutralization, respectively (although acknowledged to be very small trial) https://doi.org/10.1056/NEJMoa2102214; other vaccines show varying low levels of efficacy in preventing clinical COVID-19 but all seem to prevent severe disease. https://doi.org/10.1056/NEJMc2100362
7) Good news: the network across Africa has expanded to 40 centers; Bad news: 90% of infections in Africa are due to VoCs or variants of interest. (Wilkinson, et al., *in preparation* and https://doi.org/10.1016/S2666-5247(20)30117-8)

Session 2: Assessing immunological implications of VoCs in South Africa

This session was presented by Prof. Penny Moore, PhD, of the National Institute for Communicable Diseases of the National Health Laboratory Service, Senior Scientist in Virus-Host Dynamics at the Centre for the AIDS Programme of Research (CAPRISA) and Research Chair of Virus-Host Dynamics at the University of the Witwatersrand. Moore described the immune evasion of the 501Y.V2 variant from antibodies elicited by infection and vaccination, and the immune response to the 501Y.V2 variant. The approach was based upon years of experience with HIV and utilized the existing dynamic research network in South Africa.

Summary:

1) Structural modeling rapidly identified that 501Y.V2 would be concerning due to the location of mutations in both the NTD and RBD.
2) Re-purposed a HIV-neutralization assay for these studies which utilized a lentivirus backbone with codon-optimized spikes (wild type (WT) D614G, RBD triple mutant and full 501Y.V2) and an ACE-2 over-expressing 293T cell line (293T/ACE2.MF, courtesy M. Farzan, Scripps Research). https://doi.org/10.1038/s41591-021-01285-x
3) Comparability of data was addressed by running inter-laboratory comparison of 35 samples with David Montefiori's lab at the Duke Human Vaccine Institute and participating in the SARS-CoV-2 Neutralizing Assay Concordance Survey (SNACS); internal controls are well-characterized monoclonal antibodies.

4) The 501Y.V2 variant exhibited complete escape from neutralization by Class I, Class II and NTD-directed monoclonal antibodies – all 3 considered "therapeutically relevant."
5) The 501Y.V2 variant exhibited titre-dependent loss of neutralization by WT D614G convalescent plasma (9-fold decrease; https://doi.org/10.1038/s41591-021-01285-x), ChAdOx1 (which exhibited no protection from mild/moderate disease, though protection from severe disease is not known), and Ad26.COV2.S (despite the ≥ 82% protection observed against severe disease with this vaccine).
6) Tested cross-reactivity of neutralizing antibodies from convalescent plasma of 89 individuals infected with 501Y.V2 and showed effective neutralization of WT D614G and a 3-fold *increase* in neutralization of a newly identified variant, 501Y.V3 (now identified as equivalent to the P.1 variant). https://doi.org/10.1056/NEJMc2104192
7) The cooperation among the groups in SA and availability of genomic data allowed this work to be done in under 2 months from detection (04Dec20) to data sharing via preprint (20Jan21) and yielded the recommendation that future vaccine design efforts should include viral immunogen with the potential to elicit cross-reactive antibodies.

Session 3: Immune escape and evidence for re-infection with P.1 in Brazil

This session was presented by Prof. Ester C Sabino, MD, PhD, Director of the Department of Infectious and Parasitic Diseases at the University of São Paulo Institute of Tropical Medicine. Focus was on similar data from Brazil involving immune escape and new evidence of reinfections with the P.1 variant (aka 501Y.V3) in an area (Manaus) lacking mitigation measures against the SARS-CoV-2 outbreak. The work was enabled by and built on many years of experience working with blood banks and the availability of samples banked for up to 6 months.

Summary:

1) Data from samples collected in Manaus, capital of Amazonas (population 2M), and São Paolo (population >12M) were collected and analyzed using two commercially available kits:
    a. The Abbott AdviseDx SARS-CoV-2 IgG II chemiluminescent microparticle immunoassay (CMIA) that detects immunoglobulin G (IgG) antibodies to the SARS-CoV-2 nucleocapsid (N) protein; and
    b. The Roche Elecsys® SARS-CoV-2 Antigen double-antibody sandwich assay that detects antibodies against SARS-CoV-2 using a recombinant nucleocapsid (N) antigen and monoclonal antibodies directed against the SARS-CoV-2 N protein
2) Monthly sampling (Feb-Nov 2020) indicated that >70% of the population of Manaus had been infected with SARS-CoV-2 by Sep 2020. Calculated attack rates (correcting for cases with no detectable antibody and for antibody waning) reached 76% in October 2020 as compared to São Paolo rate of 29%. https://doi.org/10.1126/science.abe9728
3) A second wave of cases in November was determined by genomic analyses to be attributable to the emergence of the P.1 variant in Manaus.

https://doi.org/10.21203/rs.3.rs-275494/v1 Additional genetic analysis and epidemiology spanning >30 institutions around the world allowed rapid characterization of the P.1 lineage and cataloged 17 acquired mutations, three within the RBD (K417T, E484K and N501Y with the latter 2 identical to those in 501Y.V2), and all associated with increased binding to hACE2 receptor which confers 1.7-2.4 times greater transmissibility. Statistical analysis also showed that prior infection with non-P.1 lineages provided 54-79% protection against the P.1 variant. https://doi.org/10.1126/science.abh2644

4) Addressed question of how to detect rates of reinfection by looking at repeat donors over time with hypothesis that reinfection would "boost" circulating IgG antibodies and yield a V-shaped time curve. A total of 240 non-vaccinated, 3-time donors (at least one time from Apr01'20 to Jun30'20 and one from Jan01'21 to Mar31'21) were studied and data were stratified as follows with preliminary analysis predicting the chance of becoming infected with the P.1 variant:

Groups	N	Rules	P.1 infection likelihood
1 Persistently seronegative; no evidence of infection	72	No results >0.49 S/C	40.5%
2 Primary infection by P.1	49	All results ≤ 0.49 in periods 1 and 2; at least one result > 0.49 in period 3	24.3%
3.1 Primary infection by WT	68	At least one result > 0.49 in period 1 or 2; all results ≤ 0.49 in period 3	
3.2 Primary infection by WT; reinfection by P.1 unlikely	29	One result > 0.49 in period 1 or 2; antibody level in period 3 compatible with expected decline	
4.1 Primary infection by WT; suggestive of reinfection by P.1	9	One result > 0.49 in both periods 1 and 3; intermediate result with value below these two readings (V-shaped S/C time series)	10.0
4.2 Primary infection by WT; probable reinfection by P.1	13	One result > 0.49 in periods 1 or 2; antibody level in period 3 incompatible with the expected rate of antibody decline	24.3%

5) Sera from individuals previously infected with (non-P.1) SARS-CoV-2 do not cross-neutralize 2 isolates of the P.1 variant as shown by Plaque Reduction Neutralization (PRNT) assays conducted at UNICAMP.

6) Sera from individuals vaccinated with CoronaVac (inactivated viral vaccine by Sinovac) do not cross-neutralize the P.1 variant as shown by Plaque Reduction Neutralization (PRNT) assays and Virus Neutralization Test (VNT).

7) At the University Hospital of São Paolo, ~30,000 Healthcare workers received CoronaVac vaccine which is demonstrating ~50% protection even from P.1 variant starting 2 weeks after the 2nd dose.

Session 4: Impact of B.1.1.7 on vaccine induced immune responses

This session was presented by Prof. Ravindra "Ravi" Kumar Gupta, PhD, of the Cambridge Institute of Therapeutic Immunology and Infectious Disease at University of Cambridge, and the Africa Health Research Institute. Focus was on vaccine-induced immunity against the SARS-CoV-2 variant B.1.1.7 (aka "UK" and "501Y.V1") and data that identify the deletion ΔH69/V70

as a likely "permissive mutation" that allows epidemic persistence and acquisition of additional escape mutations.

Summary:

1) Global mutation rate through end of 2020 estimated at 2 amino acid changes per month; typical for a virus with short incubation period and asymptomatic transmission which contribute to relatively low selection pressure.
2) By mid-year chronic infection/shedding was recognized; immunosuppressed or persons taking cytotoxic or B-cell depleting drugs (e.g., rituximab) were accumulating mutations; key features included Spike RBD mutations (e.g., D796H) and NTD deletions (e.g., ΔH69/V70) and led to the hypothesis that such individuals were the sources of VoCs. https://doi.org/10.1038/s41586-021-03291-y
3) Characterization: Variant B.1.1.7 shares the RBD N501Y mutation seen in B.1.351 and P.1 whilst ΔH69/V70 may be a "fitness enhancer" as it increases infectivity and compensates for decreased infectivity conferred by N501Y (NB: ΔH69/V70 is also present in B1.375 lineage in the US and appears to enhance transmissibility in Syrian hamsters). B.1.1.7 has an additional deletion, ΔY144, which eliminates an antibody target, is potentially an escape mutation. Other mutations are under study. https://doi.org/10.1101/2020.12.14.422555
4) Phylogenetics; the VoCs can be named N501Y.V1 (UK, B.1.1.7), N501Y.V2 (South Africa, B.1.351), and N501Y.V3 (Brazil, P.1); target failure (or any molecular surrogates) must be coupled with sequencing to correctly identify any variants.
5) Immune Profiling: NTD and RBM (receptor binding motif) monoclonal antibody binding studies showed reduced binding (i.e., escape) whilst RBD monoclonal antibodies that were non-RBM targeting retained full neutralizing activity. https://doi.org/10.1038/s41586-021-03412-7
6) Pseudovirus (HIV particles coated with various S-glycoproteins) infectivity (i.e., entry efficiency) assays showed decrease of D796H, increase of ΔH69/V70, and compensation (restoration) towards WT levels of infectivity with presence of both mutations.
7) Serological studies of BNT162b2 ("Pfizer") vaccinees showed reduced efficacy against B.1.1.7 (3-fold after 1st dose, 2-fold after 2nd dose versus WT) and identified and age-correlated heterogeneity vulnerable population (>80 yrs of age) indicating requirement to deliver the 2 dose regimen at 3 week interval rather than 12. Convalescent sera showed slightly greater (4.5 fold) reduction in neutralization.
8) Pseudovirus assays were confirmed later by live virus assays – time is kay factor and any approach must be standardized
9) Real world vaccine efficacy reports are difficult to interpret due to varied studies that are not directly comparable. Evolution and acquisition of additional escape mutations (e.g., E484K) continue to challenge us.

Session 5: Understanding SARS-CoV-2 variants in 2021: Indian Perspective

This session was presented by Prof. Anurag Agrawal, MBBS, PhD, Director of the Institute of Genomics and Integrative Biology (IGIB) at the Center for Translational Research (CSIR). Acknowledging that much of the work leaves us with more questions than answers, focus was on trying to create common language and standards.

Summary:

1) "Double Mutant" or "Indian Mutant" → *B.1.617 Lineage* first seen in Dec 2020 with multiple sequences (55% from India; UK following); not a "double mutant" but rather 15 lineage defining variations.
2) Dec2020-Mar2021 correlation in Maharashtra between surge in cases and prevalence of B.1.617 (reaching 60-80%).
3) L452R and E484Q are dominant escape mutations that impact the hACE2 interface, increase transmissibility and confer resistance to certain monoclonal antibodies and convalescent sera (and gave rise to "double mutant" nomenclature).
4) Genomic studies also show characteristic P681R mutation near S1-S2 cleavage site that may facilitate cleavage to active S1/S2 configuration and increase infectivity. https://doi.org/10.1101/2020.12.13.422567 and https://doi.org/10.1101/2020.12.31.425021
5) Genomic surveillance in early 2021 of outbreaks across India, and in particular, Punjab, revealed virtually no B.1.617 and a vast predominance of B.1.1.7 with strong conservation across large geographical areas; indicates lack of precautions and has important public health implications.
6) Genomic surveillance also showed greater numbers of mutants in states with lower increases in number of cases.
7) While Genomic surveillance is useful, a global, integrated approach with other types of assays as described earlier, must be utilized.

Panel discussion

A panel discussion, led by Karen Makar, included the following topics and key points:

A. *What can other countries learn from the South African example about how to connect surveillance sequencing and epidemiology rapidly and efficiently with immunological assessment to inform vaccine design and usage?* [Comments from Tulio and Penny]
   - Cross-discipline and multi-institutional cooperation with government support is crucial; leveraging existing trust, knowledge and networks also key; academic, "open access" sharing also contributed to success

B. *How can we best harmonize neutralization and binding assays and incorporate international standards to improve our ability to interpret immune escape data generated in different labs and geographies?* [Comments from Karen]

- BMGF is launching GIISER (Global Immunology & Immune Sequencing for Epidemic Response) to strengthen capacity for viral immune monitoring in LMICs and build on the global networks (see slide for details)

C. *What is needed to enable more countries to generate interpretable data on immune escape more rapidly and thereby inform local and global decisions?* [Comments from Ester]

- Centralizing data and equal dissemination would be helpful
- Connecting genomics to clinical response in near-real time
- Use of internal controls and comparative efforts
  i. SNACS Concordance Survey
  ii. International Reference Standards from NIBSC (https://www.nibsc.org/science_and_research/idd/cfar/covid-19_reagents.aspx)

D. *What other in-country efforts and data are needed to understand the implication of emerging variants for vaccines, specifically?* [Comments from Anurag, Tulio]

- Agreed that what is currently going on with respect to rapid and complete sharing of research and assays is foundational
- Elevating vaccine trial data sharing to same level would also support greater speed and success against the pandemic

E. *Much of the focus on immune escape has centered on neutralizing antibody responses. For informing vaccine-related decisions, is this the appropriate focus?* [Comments from Karen, Ravi, Penny]

- How can we effectively layer in immunology on top of the sequencing and epidemiology…all concurred that applying WHO international standard might be one approach; sharing monoclonal antibodies might help (and is “easier” than sharing sera)

F. *For J&J vaccine, despite large drop in neutralizing antibody (nAbs) titres against 501Y.V2, vaccine efficacy is > 60%. Does this suggest a stronger role for CMI responses for protection (vs. nAbs)?* [Question to Penny in Q&A chat]

- Cellular responses are playing a key role in preventing severe infection – even with mRNA vaccines but trials are not constructed to enable easy comparability
- A correlate of protection and a better understanding of T-cell escape would be very useful

G. *Durability of response…How many months the individuals were "protected" for the first wave antibodies?* [Question to Ester in Q&A chat]

- Not enough numbers are in yet and no cohorts have been studied for long enough to allow proper answer.

Part II: Contribution of international collaborations and consortia to the global effort

Session 6: WHO framework for response to new COVID vaccines

This session, presented by Dr. Sylvie Briand, MD, MPH, PhD, Director of Global Infectious Hazards Preparedness (GIH) of the Health Emergencies Programme at WHO, is a description of the framework around and the decision-making group responsible for the COVID-19 global response.

Summary:

1) Graphic (see slides) of timeline spanning 2000-2020 showing major epidemic threats correlated with international collaborative efforts; founded on the 2005 publication of International Health Regulations (IHR), the *overarching legal framework that defines countries' rights and obligations in handling public health events and emergencies that have the potential to cross borders*; culminating with 2020 establishment of the Access to COVID-19 Tolls (ACT) Accelerator.
2) Defined the role of WHO as the global monitor of all SARS-CoV-2 variants of interest or concern and the coordinator of global health action.
3) Vaccines failed to mitigate the pandemic as VoCs became game-changing; nomenclature of VoCs should not be based on country of origin, but no convention has yet been adopted.
4) Monitoring & Surveillance: Key questions remain: what are the VoCs? How best to detect & monitor? Impact on transmissibility & disease severity? Impact in different risk groups? Key challenges remain: reliable, standardized methods, coordination of data, and ability to perform all such assays even in LIMCs.
5) Evidence & Assessment: Vaccine composition is one pivotal topic with a dedicated forum – currently no recommendation to change the antigen, but under constant evaluation. Drafting the framework for "CASDE" – COVID-19 Antigen Selection, Development and Evaluation. [See also: http://dx.doi.org/10.1136/bmjgh-2020-003699 for WHO-Integrate COVID-19 describing the WICID framework for decision making regarding non-pharmacological interventions].
6) Policy: updated recommendations needed to respond to VoC-changes to landscape; two bodies charged with advising include the STAG-IH (Strategic and Technical Advisory Group for Infectious Hazards) and the Strategic Advisory Group of Experts on Immunization with a COVID Vx Working Group.

Session 7: Pulling it all together in the COG-UK

This session, presented by Prof. Sharon Peacock, CBE, FMedSci, Executive Director and Chair of the COVID-19 Genomics UK (COG-UK) Consortium and Professor of Public Health and Microbiology in the Department of Medicine at the University of Cambridge, is a description of the work done in the UK to "connect the dots' of sequencing, epidemiology, virology and

immunology in a way that rapidly generates actionable information on the immunological consequences of SARS-CoV-2 variants.

Summary:

1) An organizational chart of an "hypothetical national blueprint" depicting workstreams needed to conduct appropriate and effective management of public health emergencies provided the context into which COG-UK fits as the component responsible for obtaining patient samples and sequencing the virus.
2) Formed Apr2020, 16 regional sequencing sites, 4 public health agencies and a single, centralized administration hub comprise COG-UK.
3) Local hospital labs conduct PCR testing; samples go to regional sequencing sites in a semi-random manner to control for bias and flexibility is maintained to adequately resource and respond to surges.
4) Hallmarks for success included structural pillars of in-country scientific expertise, government support, scientific leadership and functional pillars including strong decisions favoring pace over perfection, an ability to change rapidly in response to new information, a willingness to give everything away and cooperate across scientific disciplines.
5) While there is strong infrastructure in the UK, this approach may not be possible in LIMCs, so emphasis should be on testing and epidemiological surveillance together with global data concerning vaccine efficacy (see slides).

Session 8: CEPI's Agility Program: a centralised approach to evaluate VoCs

This session, presented by Dr. Simon Funnell, PhD, Scientific Leader at Public Health England (PHE; known as the UK Health Security Agency [UKHSA] effective April 1, 2021) and on the behalf of all members of the program, was a description of the Agility program instituted by CEPI to provide global centralization of data enabling evaluation of SARS-CoV-2 VoCs and assessment of their biological impacts.

Summary:

1) Within the overarching global COVAX Programme, CEPI has formed a partnership with Global Initiative on Sharing Avian Influenza Data (GISAID), the latter having created a global repository for SARS-CoV-2 sequencing data. The "Agility Program," which includes UKHSA, NIBSC, and the WHO was incepted to enable collation of data on populations (epidemiology), neutralization assays, and animal model studies.
2) Agility Program aims are to:
    a. allow rapid identification and isolation of new VoCs
    b. standardize neutralization testing using a panel of sera and the WHO antibody reference standard (20/136)
    c. enable timely reporting and information sharing
    d. enhance predictability and comparability using standardized methods in 2 parallel laboratories (CEPI and NIBSC)

3) Four steps comprise the overall approach: propagate the identified variants isolated from clinical samples in Vero/hSLAM cells and create stocks, sequence each, conduct *in vitro* neutralization studies and, if indicative of a VoC, conduct *in vivo* testing of pathogenesis and cross-protection (in a hamster model). (NB: Vero/hSLAM cells have demonstrated exceptional genomic fidelity of stock isolated and propagated within them.)
4) The NIBSC panel thus far used pre-dates July 2020 and the emergence of B.1.1.7; the WHO international standard (IS), was released in July 2020; behavior of NIBSC Agility panel and WHO IS are similar towards B.1.1.7 and P.1. The IS (blended sera) appears slightly more potent towards B.1.351 than most of the sera; comparison of growth characteristics among several VoCs also indicated more aggressive growth of focus forming units of B.1.351.
5) Challenges to being "Agile" include the need to source clinical material containing culturable virus from all parts of the globe, reliance on international collaborations, and hinderances by Material Transfer Agreements (MTA) and regulatory shipping restrictions.

Session 9: Production of SARS-CoV-2 stocks

This session, presented by Dr. Sujatha Rashid, PhD, Program Manager of Biodefense and Emerging Infections Research Resources Repository (BEI Resources), which is funded by NIIAID and managed by the American Type Culture Collection (ATCC), focused on the importance of minor variant analysis by NGS to qualify SARS-CoV-2 stocks and described the resources and activities around SARS-CoV-2 variant analysis available through BEI Resources.

Summary:

1) BEI Resources was created in 2003 partially in response to the SARS outbreak with the stated mission to centralize "the acquisition, authentication, production, preservation, storage, and distribution of a broad range of unique and quality assured research materials for the infectious disease research community," and to "aid in the development and evaluation of vaccines, therapeutics, and diagnostics."
2) For SARS-CoV-2, BEI Resources has distributed isolates from early days of the pandemic and has become the central repository in the US for emerging variants. Other activities include optimizing methods for virus propagation, developing sequencing tools for qualifying virus stocks and supporting the scientific community by providing >170 unique products.
3) Into late 2020, propagation up to liters of virus stocks was predominantly in Vero E6 line. Collaborators reported unexpected results with certain stocks of the Washington 1 isolate used in animal studies leading to development of a pipeline for analyzing next-generation sequencing (NGS) data for low frequency changes. This revealed adaptive point and deletion mutations, specifically in the furin cleavage site of the spike. Changes accumulated through additional passages and were confirmed by researchers producing their own virus stocks. Recent research corroborates the role of the furin cleavage site mutation. https://doi.org/10.1038/s41586-021-03237-4
4) Discrepancies in virus stocks propagated using same seed virus pointed to heterogeneity of ACE2 receptor density of the Vero E6 line as the potential cause.

5) Animal studies indicated a critical role of the furin cleavage site in SARS-CoV-2 pathogenesis. This led to the use of Vero lines over-expressing the serine protease *TMPRSS2*, which reportedly reduces selection of the spike adaptive mutants.
6) BEI Resources is now propagating SARS-CoV-2 stocks in Calu-3, a human lung adenocarcinoma (anchorage-dependent) cell line with high endogenous ACE2 that displays greater viral genome integrity with lower frequency of point mutations when compared to virus produced in Vero cell lines. The downside of Calu-3 is that expansion is slow, frequent media changes are required, plaque assays are difficult to establish, and there is similar issue with heterogeneity of cell banks.
7) BEI Resources finds Calu-3 to be a better choice for virus production. The role of variant analysis using NGS was presented using B.1.351 isolate (from Alex Segal) and a B.1.1.7 isolated from same clinical sample in different cell lines (from CDC and UC San Diego).

Session 10: SARS-CoV-2 Interagency Group (SIG) Variant Assessment / Characterization

This session, presented by Dr. Steve Oberste, PhD, Chief, Polio and Picornavirus Laboratory Branch of the Division of Viral Diseases at the National Center for Immunization and Respiratory Diseases (NCIRD) of the Center for Disease Control and Prevention (CDC), was a description of the structural framework around and the decision-making groups responsible for the US COVID-19 response in the context of the global pandemic and the functional domains responsible for conducting analyses.

Summary:

1) The mission and goals of the SIG are to monitor and assess impact of SARS-CoV-2 variants on vaccines, therapeutics, diagnostics and public health control efforts with the overarching remit to foster interagency coordination and cooperation and to support appropriate, defensible and timely public health measures including effective communications.
2) In silico, *in vitro* and *in vivo* approaches are coordinated and reported to the SIG Technical Working Groups and feed into SIG assessments and decision making.
3) Variant analysis work streams for vaccines include surveillance, observational studies, vaccine companies, genomic data and antibody escape mapping.
4) Variant testing pipeline and classification runs from identification of a variant containing a mutation of concern → variant of interest → variant of concern → variant of high consequence (i.e., evades all known mitigations). See slides for more detailed definitions. Speed is dependent on developing reliable methods to assess the impact of each mutation.
5) There is ongoing global cooperation and coordination to identify and track variants, standardize assays, develop best practices and ensure alignment and equitable distribution of resources.

Session 11: ACTIV / TRACE: OpenData portal

This session, presented by Dr. Christine Colvis, PhD, Director of Drug Development Partnership Programs at the National Center for Advancing Translational Sciences (NCATS) of the NIH, was a description of a public private partnership (PPP) comprised of pharmaceutical companies and government agencies and which complements the SIG described in Session 10.

Summary:

1) This PPP, Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV), was formed in April 2020 *to develop a coordinated research strategy for prioritizing and speeding development of the most promising treatments and vaccines*.
2) The Tracking Resistance and Coronavirus Evolution (TRACE) working group is charged with:
    a. developing processes and infrastructure for monitoring and testing emerging SARS-CoV-2 variants
    b. gathering, standardizing, and sharing variant sequencing data as well as data on changes in therapeutic or vaccine efficacy due to changes in the viral sequence
3) TRACE utilizes a 5-step workflow that emphasizes speed and collaboration to achieve a comprehensive report that is publicly available.
4) National Center for Biotechnology Information (NCBI) is currently making sequencing data available at: https://ftp.ncbi.nlm.nih.gov/pub/ACTIV-TRACE/ and will soon launch an interactive website for accessing and interacting with the sequencing data.
5) The OpenData Portal / Variant Therapeutic Pages has launched a Minimal Viable Product (MVP) that includes Variant Therapeutic Data Summaries, a Variant Dataset Browser and Therapeutic Assay Overviews at: https://opendata.ncats.nih.gov/covid19/

Panel discussion

A panel discussion, led by Bill Dowling, included the following topics and key points:

- *Comments by Dr. Divya Shah, PhD, Epidemics Research Lead at the Wellcome Trust were invited. Divya explained that the Trust is actively seeking partnerships and offering funding on a number of fronts, including support for research and development efforts towards new vaccines and treatments for COVID-19. Now focusing on a hub-and-spoke model to build a network that can conduct genomic surveillance and perform bioinformatics analysis, and engage with policymakers. Wellcome is assessing needs, be they human or technical resources, financial support, training, etc. Wellcome are encouraging that sequence data is linked with associated clinical and epidemiological data to be more meaningful and shared with Public Health agencies to enable a rapid, integrated, global response. Efforts will begin across Africa (Malawi, Kenya, South Africa, Gambia, and Uganda) and parts of Asia (Thailand, Vietnam and neighboring countries). This work will feed into our longer thinking at Wellcome and is intended to extend beyond COVID-19 in order to stay prepared for future global health threats.*

- *What opportunities are there for scientists to contribute data on the impact of SARS-CoV-2 variants to international efforts? How do they feed into the various and vast resources (i.e., who to call)?*
    - [Steve] CDC maintains close contact with State Health Departments; linking directly with academics is a bit more difficult but contact through "CDC info" is recommended; also the NIH has open lines to grantees.
    - [Sharon] Data in GISAID is an open access database that is continuously growing; a network of academic investigators and collaborators are continuously working on the data; COG-UK can offer sequencing advice and potentially support on a global level
- *How can we best harmonize neutralization and binding assays and incorporate international standards to improve our ability to interpret immune escape data generated in different labs and geographies?*
    - [Simon] Given availability of the International Standards, the global community should adopt their use. NIBSC and other sources also offer research panels of sera for assay development purposes which should reduce the requirement on the IS. Converting titres into International Units is likely to become a requirement for vaccine developers and vaccine assessment organisations thus enabling comparison of data sets for each variant. (Simon offered plaudits to CEPI and the WHO for the weekly teleconferences on all aspects of assay development, models of infection and the various Solidarity trials with literally hundreds of participants attending each forum.)
    - [Steve] Stressed comparability by using panels. Challenges exist for collecting enough samples; for linking a given sample with a particular virus (acute phase is long gone)
    - [Bill] pointed out availability of standardization materials that are available from government agencies and offered to assist anyone interested in obtaining them (simply email or call him)
- *What resources can be made available to the international scientific community in order to identify and characterize new variants (reagents, standards, protocols, assays, data)?*
    - [Sujatha] BEI Resources is working to have at least one good representative of each emerging variant available in the catalog. The program can also support cell-based assay development. Cell lines and sera are also available. Protocols and technical support are available (Knowledge Base section of website).
    - [Simon] CEPI, through UKHSA and NIBSC, is making variant stocks as available as possible through depositing stocks in BEI Resources, NIBSC and The European Virus Archive.

Wrap-up and next steps

Dr. Ivana Knezevic thanked Karen, Bill and presenters as well as attendees for their participation in the workshop and provided the following comments:

A. It was good to hear and understand the needs and desired focus of efforts going forward. So many important issues and great questions.

B. Take-aways:

- The significance of work within individual countries or regions (i.e., local efforts) cannot be overstated. The speed of science at which the network in South Africa pivoted the vaccine world is unprecedented. Given that the relatively new tool of NGS is important (and perhaps essential), in the end, it was openly sharing scientific insights and the immunological studies of vaccine and convalescent SARS-CoV-2 sera that combined to allow rapid characterization of the VoCs as well.
    - USG and UK can prioritize based on the biggest threats within respective geographies and get "ahead of the curve" with massive vaccine rollouts.
    - Immune monitoring in LMICs, where vaccine rollout is either delayed or not possible, takes on increased importance as the virus has more time to mutate; these become the key areas to monitor for emerging variants
    - Real-time response is no longer a "nice-to-have" but rather a need and should become a priority as we seek true epidemic preparedness innovations.
- This translates or escalates to the global need for rapid sequencing, technical support in some cases, and a centralized database to support the most rapid and effective response to emerging variants in the context of a pandemic.
- Use of WHO International Standard in neutralizing assays should be encouraged to enable harmonization and comparability of analyses.
- Global leadership by WHO should continue and WHO encourages active engagement by all members of the global community.

C. The COVAX Enabling Sciences SWAT plan to continue sharing learnings and may plan future workshops.

D. Resources are shared here: https://epi.tghn.org/covax-overview/