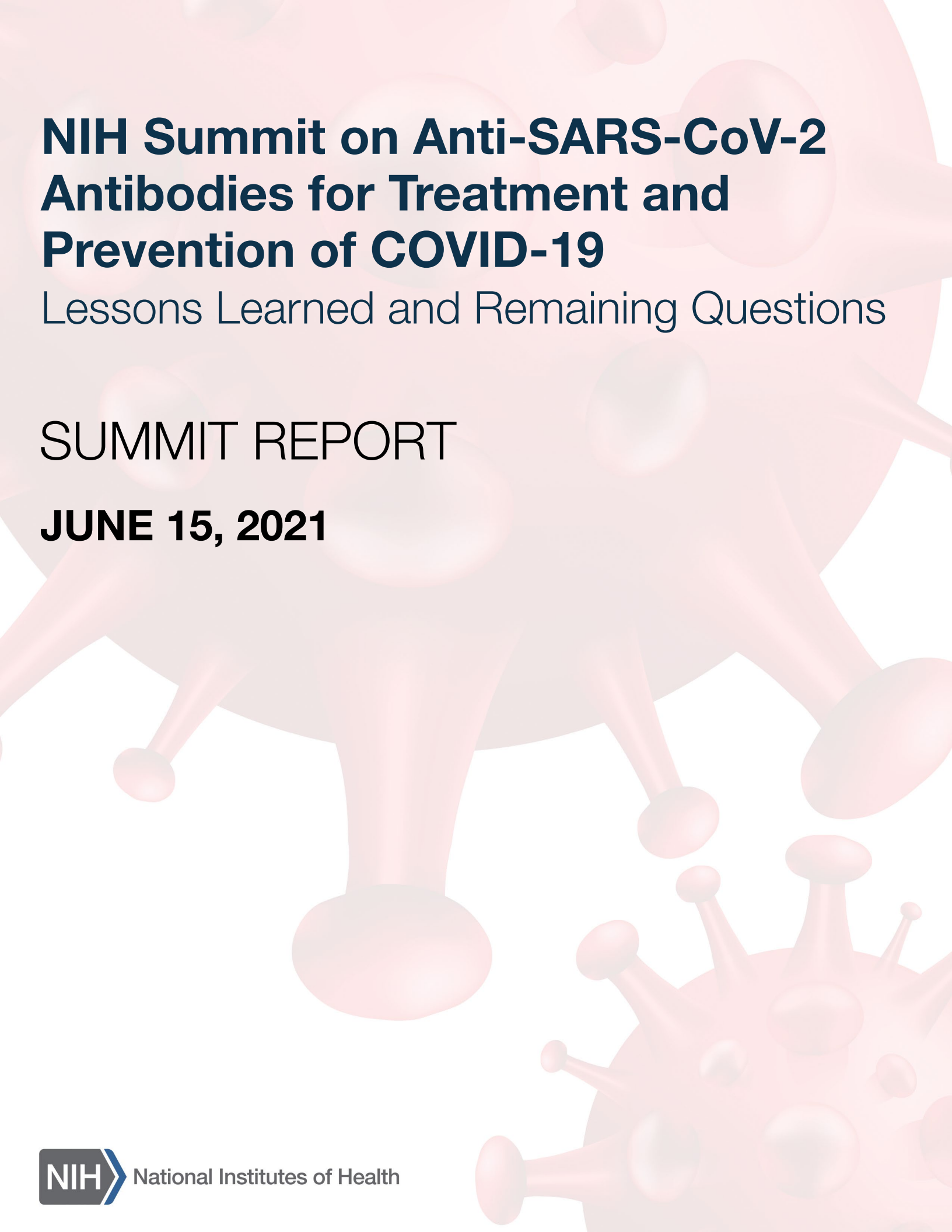
NIH Summit on Anti-SARS-CoV-2 Antibodies for Treatment and Prevention of COVID-19

Lessons Learned and Remaining Questions

SUMMIT REPORT

JUNE 15, 2021



National Institutes of Health

Summit Report Authors and Contributors

César Boggiano, Ph.D., National Institute of Allergy and Infectious Diseases, NIH
Robert W. Eisinger, Ph.D., Office of the Director, NIH
Andrea Lerner, M.D., National Institute of Allergy and Infectious Diseases, NIH
James M. Anderson, M.D., Ph.D., Office of the Director, NIH
Katharine Bar, M.D., University of Pennsylvania
Ralph Baric, Ph.D., University of North Carolina at Chapel Hill
Kara Chew, M.D., M.S., University of California, Los Angeles
Myron S. Cohen, M.D., University of North Carolina at Chapel Hill
Stephanie L. Courchesne, Office of the Director, NIH
Rajesh Gandhi, M.D., Massachusetts General Hospital
C. Taylor Gilliland, National Institute of Biomedical Imaging and Bioengineering, NIH
Bette Korber, Ph.D., Los Alamos National Laboratory
Jens Lundgren, M.D., D.M.Sc., Rigshospitalet, University of Copenhagen
Peter Marks, M.D., Ph.D., U.S. Food and Drug Administration
Adrian McDermott, M.Sc., Ph.D., National Institute of Allergy and Infectious Diseases, NIH
Katelynn A. Milora, Office of the Director, NIH
Erica O. Saphire, Ph.D., La Jolla Institute for Immunology
Connie Schmaljohn, Ph.D., National Institute of Allergy and Infectious Diseases, NIH

Summit Hosts

Francis S. Collins, M.D., Ph.D., National Institutes of Health (NIH)
Janet Woodcock, M.D., U.S. Food and Drug Administration
Anthony S. Fauci, M.D., National Institute of Allergy and Infectious Diseases, NIH

Summit Organizers

James M. Anderson, Office of the Director, NIH
César Boggiano, National Institute of Allergy and Infectious Diseases, NIH
Robert W. Eisinger, Office of the Director, NIH
Andrea Lerner, National Institute of Allergy and Infectious Diseases, NIH
C. Taylor Gilliland, National Institute of Biomedical Imaging and Bioengineering, NIH
Stephanie L. Courchesne, Office of the Director, NIH
Katelynn A. Milora, Office of the Director, NIH
Elizabeth Wilder, Office of the Director, NIH
Stacey Adam, Foundation for the National Institutes of Health
Kevin Bugin, U.S. Food and Drug Administration
Alison Deckhut, National Institute of Allergy and Infectious Diseases, NIH
Nahed El Kassar, National Heart, Lung, and Blood Institute, NIH
Alan Embry, National Institute of Allergy and Infectious Diseases, NIH
Karl Erlandson, Biomedical Advanced Research and Development Authority, Department of Health and Human Services
Stacy Ferguson, National Institute of Allergy and Infectious Diseases, NIH
Robert Fisher, U.S. Food and Drug Administration
Simone Glynn, National Heart, Lung, and Blood Institute, NIH
Matthew Hall, National Center for Advancing Translational Sciences, NIH
Libby Higgs, National Institute of Allergy and Infectious Diseases, NIH
Amy Jenkins, Defense Advanced Research Projects Agency
Nicole Kilgore, Department of Defense
Mary Marovich, National Institute of Allergy and Infectious Diseases, NIH
Amy Palin, National Institute of Allergy and Infectious Diseases, NIH
Amy Patterson, National Heart, Lung, and Blood Institute, NIH
Jean Patterson, National Institute of Allergy and Infectious Diseases, NIH
Steve Smiley, National Institute of Allergy and Infectious Diseases, NIH
Randall Tressler, National Institute of Allergy and Infectious Diseases, NIH
Carol Weiss, U.S. Food and Drug Administration
Catherine Yen, National Institute of Allergy and Infectious Diseases, NIH

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Introductory Comments

Francis S. Collins, M.D., Ph.D., Director, National Institutes of Health (NIH); Janet Woodcock, M.D., Acting Commissioner, U.S. Food and Drug Administration (FDA); and Anthony S. Fauci, M.D., Director, National Institute of Allergy and Infectious Diseases (NIAID), NIH

Dr. Francis S. Collins welcomed participants to the third in a series of summits addressing anti-SARS-CoV-2 antibodies. He commented that although several vaccines are available and some monoclonal antibodies (mAbs) have received Emergency Use Authorization by the United Sates Food and Drug Administration (FDA) for outpatient settings, the emergence of variants has caused researchers to rethink how to proceed in the development and use of anti-SARS-CoV-2 antibodies for treatment and prevention of COVID-19. Dr. Collins stated the purpose of this summit is to provide an overview of the current knowledge and lessons learned on clinically relevant anti-SARS-CoV-2 antibodies and to identify key unanswered scientific questions to catalyze antibody clinical development and implementation. He emphasized that the presentations and discussions at this Summit will inform the future directions in this field. Dr. Janet Woodcock emphasized the importance of synthesizing what has been learned about passive immunity in the setting of the COVID-19 pandemic and determining future directions. As the pandemic continues to spread globally and variants of concern (VoCs) emerge in much of the world, antibody therapy will continue to be one of the important tools used for both prevention and treatment of COVID-19. Dr. Anthony S. Fauci noted the successful use of mAbs in preventing the progression of hospitalizations if given early enough. He commented that this summit will begin to identify key scientific questions that will inform future research, development, and optimal use of these antibodies.

César Boggiano, Ph.D. (NIAID, NIH) provided an overview of the agenda for today's Summit and moderated the meeting. Dr. Boggiano introduced the first group of presenters to provide the foundation for the Summit with a series of state of the science presentations.

State of the Science Presentations

Convalescent Plasma and Hyperimmune Globulin

Peter Marks, M.D., Ph.D., FDA

Dr. Peter Marks presented on the use and potential efficacy of convalescent plasma (CP) and hyperimmune globulin (HIG) for COVID-19. He noted that the rationale for use of CP as treatment of respiratory viruses was based on its use as treatment in previous influenza and coronavirus outbreaks. Generally, the best efficacy was seen when CP with known high antibody titers was used, and the greatest improvement in mortality was seen when CP was administered early after symptom onset. These beneficial effects were reported in literature as early as the 1950s.

Following the initial SARS-CoV-2 outbreak in China, several studies were conducted on the use of CP for treatment of COVID-19 including randomized controlled trials, controlled trials based on plasma availability, retrospective matched cohort studies, and case series. Findings from a retrospective matched cohort study showed patients treated with CP soon after admission showed some survival benefit compared to those who were administered CP later during the disease course.[1] Several randomized trials in hospitalized patients with more advanced disease who were intubated or about to be intubated showed no beneficial effects of CP, except for those patients who were immunocompromised. Additional trials reported efficacy in earlier stage disease, suggesting that although CP treatment does not benefit those patients with advanced COVID-19, the treatment may benefit those individuals during the early stages of illness.

Dr. Marks described the U.S. CP Expanded Access Protocol (EAP) sponsored by the FDA and the Biomedical Advanced Research and Development Authority in the U.S. Department of Health and Human Services and led by the Mayo Clinic (NCT04374370). EAP was a single-arm clinical protocol that treated more than 70,000 patients at more than 2,700 sites. Although patients received many concomitant treatments over the course of the study—complicating identification of a signal—the EAP found that accurate determination of antibody titer was essential because clinical benefit for the previously indicated patient population was only observed when using high antibody titer CP. Using the Broad Institute of MIT and Harvard biosafety level 3 (BSL-3) neutralization assay, EAP patients were divided into higher and lower titer groups. This analysis showed a modest absolute improvement in survival for those treated with CP at an earlier stage of disease: relative improvements at 7 days were 10% to 15% in mortality rates.

Dr. Marks commented that high-titer versus low-titer CP was associated with a modest survival benefit that was most apparent when administered early after symptom onset. He emphasized that these findings had similarly been observed with other viral infections.

Dr. Marks' presentation also addressed the use of HIG for treatment of COVID-19. Clinical trials evaluating HIG for treatment of COVID-19 began relatively late in the pandemic. Initial studies of HIG were conducted in hospitalized patients with moderate to severe disease, but no clear benefit was shown in at least one randomized clinical study. Studies of HIG in the outpatient setting are ongoing currently.

Dr. Marks concluded his presentation by noting that that CP appears to act like a conventional antiviral, so benefit may only be seen when administered early in the course of the disease, with the appropriate potency and high titer antibodies.

Monoclonal Antibodies for Prevention of COVID-19

Myron S. Cohen, M.D., University of North Carolina at Chapel Hill

Dr. Myron Cohen presented on the decades of scientific development that provided the scientific foundation for the use of mAbs in the prevention and treatment of COVID-19. He noted that many clinical researchers working in related fields rapidly pivoted to contribute to COVID-19 research and treatment efforts. U.S. government investments in the COVID-19 response in 2020 included Operation Warp Speed; the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) program;[2] and the formation of the COVID-19 Prevention Network, in which several NIAID networks previously working in other clinical research areas collaborated to conduct phase 3 clinical trials on treatments and preventative measures for COVID-19. These activities worked synergistically on vaccines and mAbs and represent the contributions of thousands of study participants, clinical trial sites, investigators, and industry partners.

Dr. Cohen noted that several companies already were working on mAbs early in the pandemic, with Eli Lilly and Company's (Lilly) and Regeneron's representing the most advanced efforts. As of June 2021, the FDA has granted emergency use authorization (EUA) to several mAbs to treat early COVID-19 including: Lilly's bamlanivimab, which was withdrawn subsequently as a single agent; a combination of bamlanivimab and etesevimab; Regeneron's combination of casirivimab and imdevimab, known as REGEN-COV; and Vir Biotechnology, Inc.'s sotrovimab. Additional mAbs are being studied, primarily as therapeutic agents. Furthermore, mAbs may be beneficial in stopping viral replication and blocking progression of disease in unrecognized asymptomatic infection, which is currently being evaluated in clinical trials.

Dr. Cohen commented that mAbs also offer immediate preventive protection for those exposed or unvaccinated in high-risk settings. mAbs can be provided to people unlikely to respond or allergic to components of a COVID-19 vaccine. mAbs also helped predict requirements for a vaccine by identifying required titers of neutralizing antibodies. Target populations for preventive use of mAbs include both residents and health care providers of nursing homes; index case contacts, such as household contacts; compromised hosts, such as people with HIV or others who are immunosuppressed; and those in high-incidence workplaces, such as meat-packing plants.

Nursing homes are particularly high-incidence areas, with nursing home residents or workers comprising one-third of all COVID-19 deaths in the U.S. When bamlanivimab became available, as part of the Phase 3 Blocking Viral Attachment and Cell Entry with SARS-CoV-2 Neutralizing Antibodies (BLAZE) -2 Post-Exposure Prophylaxis Study (NCT04497987), Lilly utilized mobile units to deliver bamlanivimab versus placebo to nursing homes experiencing outbreaks. The study was conducted in approximately 140 facilities with median participant ages of 75 to 80 years. The findings served as proof of concept for the use of mAbs, showing reduced incidence of COVID-19, reduced symptoms, and no deaths among patients who were administered bamlanivimab versus placebo.[3]

In the REGEN-COV 2069 Phase 3 clinical study (NCT04452318), the combination of casirivimab with imdevimab was administered subcutaneously to all contacts of a household in which one member had been diagnosed with COVID-19. Household contacts who received REGEN-COV showed no symptomatic cases of COVID-19 and a 50% reduction of overall rates of infection with SARS-CoV-2 compared to the placebo group. Infections occurring among participants administered REGEN-COV therapy were all asymptomatic, shorter duration and low viral loads compared to individuals in the placebo group who acquired SARS-CoV-2. This study reported its first 400 subjects as preliminary results,[4] and data from the full study of 1,000 participants is forthcoming.

Dr. Cohen noted that the detection of viral variants in those who received mAbs was rare, but possible. He emphasized that these studies demonstrate proof of concept that passive immunity works and that relevant mAbs can be made quickly.

In summary, Dr. Cohen noted that mAbs capable of neutralizing SARS-CoV-2 were generated rapidly, and EUAs have been authorized by the FDA for several agents to treat COVID-19 early in the disease course in individuals at high risk of progressing to severe disease. Two NIH–industry collaborative prophylaxis studies have shown promising results demonstrating the ability of mAbs to prevent COVID-19 when given as post-exposure prophylaxis. He commented that the effects of asymptomatic empiric treatment with mAbs in prevention trials are being evaluated. Additional study is required on the interactions between mAbs and vaccines in prevention trials. Dr. Cohen described several mAbs that have modifications to the fragment crystallizable (Fc) region, such as AZD7442 and sotrovimab, that provide a long half-life. These mAbs will likely provide critical protection from COVID-19 in long-term usage as an alternative to vaccination for those who cannot mount an endogenous immune response. While logistical challenges in the administration of mAbs for prevention remain, one potential future improvement is further exploration of subcutaneous delivery. Dr. Cohen concluded that mAbs will continue to play an important role in public health and medical management of COVID-19.

mAbs for Outpatient Treatment of COVID-19

Kara Chew, M.D., M.S., University of California, Los Angeles

Dr. Kara Chew presented on the use of mAbs for outpatient treatment of COVID-19. She described how mAbs are neutralizing human antibodies that most commonly target the S1 receptor-binding domain (RBD), inhibiting interaction of the RBD with the angiotensin-converting enzyme 2 (ACE2) receptor to block viral entry. In addition to blocking viral entry, some mAbs may enhance the clearance of infected cells through Fc-mediated effector mechanisms. Single and combination mAbs are in development, from preclinical stages to Phase 3 clinical trials. The different mAbs vary in the epitopes targeted. Epitopes may be in the ACE2 binding region, such as in the receptor-binding motif (RBM), or outside the ACE2 binding region, such as the receptor-binding core, a more conserved region less susceptible to mutations. Most mAbs are currently administered intravenously; other routes—including intramuscular, subcutaneous, and inhaled—are under evaluation. Dr. Chew commented that understanding the targets of mAbs helps identify the potential risk to their activity with variants.

Dr. Chew described the findings from several clinical studies of mAbs. In the Phase 2 BLAZE-1 (A Study of LY3819253 [LY-CoV555] and LY3832479 [LY-CoV016] in Participants with Mild to Moderate COVID-19 Illness) (NCT04427501) trial for early COVID-19, bamlanivimab IV (Lilly, Inc) was studied at a range of doses (700mg, 2800mg, and 7000mg vs. placebo). In an initial interim analysis, about 70% of participants had a risk factor for severe COVID-19, with a median of 4 days of symptoms at study entry. The primary virologic outcome was met for the 2,800mg dose of bamlanivimab and it appeared that bamlanivimab reduced viral load faster than placebo. Composite hospitalization and emergency department (ED) visit rates were lower for those on bamlanivimab (all active arms pooled) compared to the placebo arm, at 1.6 and 6.3%, respectively. This trial provided the basis for the first EUA for a mAb to treat COVID-19. Individuals who were hospitalized showed persistently high nasopharyngeal SARS-CoV-2 viral loads post-entry, whereas those who were not hospitalized showed steady declines.[5] Dr. Chew summarized subsequent data from the BLAZE-1 trial (NCT04427501) that included a bamlanivimab/etesevimab (2800mg/2800mg) combination arm in outpatients with mild to moderate COVID-19. The findings showed treatment with the mAb combination compared with placebo was associated with significant reductions in SARS-CoV-2 viral load, with no difference in viral load reduction with bamlanivimab monotherapy. Low rates of adverse events were observed across all arms. The differences in hospitalization and ED visits was particularly significant among the highest risk patients in a post-hoc analysis.[6] Overall, this mAb combination showed a 70% reduction in rates of the composite outcome of COVID-19 related hospitalization or any cause death in the Phase 3 trial among non-hospitalized COVID-19 patients compared to placebo.[7] This study also reported a modest (1 day) reduction in time to sustained symptom resolution.

Dr. Chew described the findings from the clinical trials (NCT04425629) of the mAb combination of casirivimab and imdevimab, designated as REGEN-COV (Regeneron) in outpatients. In the Phase 2 trial, two doses of casirivimab/imdevimab were tested compared to placebo among outpatients with less than 7 days of symptoms. About 64% had at least one risk factor for hospitalization. The decrease in medically attended visits for participants in both dose arms combined compared to the participants in the placebo arm (3% vs. 6%, respectively) showed a potential clinical benefit of this mAb combination.[8] The Phase 3 trial of the casirivimab/imdevimab combination compared to placebo confirmed the clinical benefit of casirivimab/imdevimab in reducing rates of hospitalizations and deaths. Dr. Chew commented that the greater reduction in symptom duration was a notable finding in this trial—symptoms were reduced by 4 days with both 1,200mg and 2,400mg doses of

REGEN-COV compared to placebo. The relative risk reduction in hospitalizations and deaths was approximately 70% for the 1200mg total dose and 71% for the 2400mg total dose.[9, 10] She noted that this level of reduction was similar to that observed in the bamlanivimab/etesevimab trials and that high viral loads persisted at followup in those individuals who later were hospitalized, regardless of baseline SARS-CoV-2 serostatus. Dr. Chew also described the casirivimab/imdevimab intravenous and subcutaneous delivery Phase 2 dose-ranging trial (NCT04666441) that showed similar viral load reductions with intravenous and subcutaneous deliveries. While all doses in this trial met their primary endpoint with viral load reductions compared to placebo, additional results are pending.

Dr. Chew described the single mAb sotrovimab (Vir Biotechnology and GSK), which was originally isolated from a severe acute respiratory syndrome (SARS) survivor. This mAb targets the RBD core and a conserved epitope and does not compete with ACE2 receptor binding. It has neutralizing activity across Sarbecoviruses, and an LS modification in the Fc domain to extend the mAb half-life. The COVID-19 Monoclonal Antibody Efficacy Trials – Intent to Care Early (COMET-ICE) Phase 3 trial (NCT04545060) evaluated 500mg intravenous sotrovimab compared to placebo among participants with at least one risk factor for COVID-19 progression and 5 or fewer days of symptoms. This trial was stopped early for efficacy as treatment with sotrovimab resulted in an 85% reduction in risk of hospitalization or death in high-risk outpatients compared to the patients in the placebo arm.

Dr. Chew summarized that anti-SARS-CoV-2 mAb therapies are safe and effective in preventing hospitalization and deaths in symptomatic SARS-CoV-2 infection when given early in the disease course. She noted that mAbs are vulnerable to virus evolution, with varying risks depending on the epitopes they target. Therapeutic options that are more stable (expected to retain) in view of variants are needed, with combination therapies and those that target conserved regions being promising. There are several mAbs singly and in combination in development and clinical trials. Clinical outcomes data using mAbs are limited currently for VoCs and variants of interest. Additionally, some risks and benefits of mAbs remain unknown, including the possibility of treatment-emergent resistance. She concluded her presentation noting that intravenous delivery of mAbs remains a challenge; however, intramuscular and subcutaneous administration, especially in the outpatient setting may be easier and feasible.

Antibody Treatment for Hospitalized COVID-19 Patients

Jens Lundgren, M.D., D.M.Sc., Rigshospitalet, University of Copenhagen

Dr. Jens Lundgren presented several clinical studies evaluating the administration of antibody treatment to hospitalized COVID-19 patients. He described the pulmonary and vascular compartments that are the sites of disease-causing viral replication; viral load in these locations is prognostic for ultimate outcome. The standard of care for such patients includes a combination of an antiviral agent, such as remdesivir, and immunomodulating agents. He commented that with certain other viral diseases, treatment with a combination of two or more antiviral agents leads to a better outcome. He described several recent clinical studies that examined whether neutralizing antibodies (NAbs), in addition to standard-of-care, are safe and may improve clinical outcomes in hospitalized patients with COVID-19. The ACTIV-3 or Therapeutics for Inpatients With COVID-19/International Network for Strategic Initiatives in Global HIV Trials (TICO/INSIGHT) 014 trial (NCT04501978), served as a platform for a Phase 3 trial of novel antiviral agents to demonstrate early safety and early futility with a day 5 ordinal scale outcome. The primary endpoint of the trial was sustained recovery during 90 days of followup. The first 3 agents evaluated in this trial (Bamlanivimab [Lilly], Sotrovimab [GSK/Vir], and Brii 196 +198 [Brii Biosciences]) failed the futility assessment and thus were subsequently unblinded. Studies of two agents,

AZD 7442 (AstraZeneca) and MP0420 (Molecular Partners-DARPin technology) are ongoing. Another trial, INSIGHT 013 trial, also known as the Inpatient Treatment with Anti-Coronavirus Immunoglobulin (ITAC) (NCT04546581), used a day 7 ordinal pulmonary outcome as its primary endpoint and tested a standardized hyperimmune gammaglobulin (CSL Behring, Emergent BioSolutions, Grifols S.A. and Takeda Pharmaceutical). This clinical study has been completed.

In both trials, COVID-19 patients hospitalized within 12 days of symptom onset were randomized to either an investigational agent plus standard-of-care arm or a placebo plus standard-of-care arm. Collectively, five antibody agents were studied, including single mAbs bamlanivimab and sotrovimab and two combinations with non-overlapping epitopes, Brii 196+198 and AZD 7442 (AstraZeneca). The fifth agent was a hyperimmune IgG (hIVIG), a standardized product that targets multiple epitopes and has been shown to be effective against all tested SARS-CoV-2 VoCs. In the clinical trial testing bamlanivimab, the active arm did not show clinical benefit compared to patients in the placebo arm. Of note, the primary endpoint was redesigned from an earlier trial because some patients were readmitted or died, so discharge was no longer considered recovery. Sustained recovery was redefined as returning to a prehospitalization residence for ≥14 consecutive days.[11] Dr. Lundgren noted that the results of the first four completed evaluations of bamlanivimab, Brii 196-198, sotrovimab, and hIVIG, showed that only the latter two agents resulted in modest and non-significant favorable outcomes compared to patients in the other treatment arms or the placebo arm. Studies of two agents, AZD 7442 and MP0420 (Molecular Partners-DARPin technology) are ongoing.

Dr. Lundgren outlined three possible explanations why antibody treatments administered to hospitalized COVID-19 patients have not proven effective to date. One explanation is that active SARS-CoV-2 replication will be stopped regardless of antibody treatment as a result of either treatment with remdesivir or the host immune response. Another possibility is that the antibody is not sufficiently active to significantly add to antiviral treatment due to lack of activity *in vivo* or rapid emergence of drug resistance. A third possibility is that the antibody has harmful effects that counteract any beneficial effects. He noted that some of these explanations may apply in some subgroups of patients and not in others.

Dr. Lundgren outlined several factors that could be considered including clinical parameters, such as duration of infection; antibodies against RBD; plasma nucleocapsid antigen; host inflammatory and coagulation markers; and viral RNA levels from nasal swabs. Data addressing these factors are currently available only for the first TICO trial, which compared bamlanivimab versus placebo. Dr. Lundgren showed the mean binding inhibition using the NAb assay in the first 5 days of treatment with bamlanivimab vs placebo normalized to baseline antibody status. He noted that those study participants randomized to placebo who were antibody-negative at study entry showed a gradual production of antibodies over time, which was less evident for those who had antibodies at entry. At study entry, patients showed a wide range of plasma antigen levels, which is a reasonable proxy for total viral replication throughout the body. Those participants with high antigen levels showed declines between day 1 and day 3 in both the treatment and placebo arms, with little antigen remaining at day 5. Some patients in the placebo group attained sustained recovery, but this was less likely for those patients who had no preexisting antibodies, those with high viral antigen levels, and those who had both. Patients with high IL-6 levels at study entry had a 50 percent lower chance of sustained recovery than those who entered the study with lower IL-6 levels.

Analysis of the data supported a pre-specified hypothesis, stating that sustained recovery after administration of bamlanivimab versus placebo differed by presence of neutralizing antibodies at study entry, especially if participants had markers of elevated viral replication.

Dr. Lundgren noted that the clinical trials, which were deployed rapidly and had a quick turnaround of results, helped inform the field on the use of these agents. He commented that bamlanivimab, sotrovimab, Brii 196+198, and hIVIG likely do not improve outcomes overall among hospitalized patients with COVID-19. AZD7442 remains under blinded evaluation following passing initial futility evaluations. He noted that there may be a benefit in subgroups with a blunted antibody response is plausible, but this remains to be confirmed. The null effect does not appear to be explained by infection with virus resistant to the NAbs or undesired change of viral replication kinetics from the use of the NAbs. He suggested that the possibility of hyperinflammation induced by NAbs requires additional investigation and confirmation.

Mutations and Variants of Concern

Bette Korber, Ph.D., Los Alamos National Laboratory

Dr. Bette Korber presented the latest information on emerging SARS-CoV-2 variants based on data from the Global Initiative on Sharing All Influenza Data (GISAID) as of June 9, 2021.[12] Almost all of the emerging variants of interest have mutations in the N-terminal domain (NTD) and Receptor Binding Domain (RBD); many also carry mutations at the furin cleavage site. She commented that the regions are evolving simultaneously, so variants often carry multiple mutations. Highly repeated mutations among many emergent lineages are localized to epitope regions. Many of these mutations have been shown to confer partial resistance to convalescent sera and neutralizing monoclonal antibodies (Nabs), indicative of immune pressure as a selective force. She noted that mutations in the RBM and near the furin cleavage site and indel patterns also may affect infectivity.

Dr. Korber described two distinct variant trajectories that have occurred in the United Kingdom and North America. In the United Kingdom, the B.1.1.7 (Alpha) variant became very common, but the B.1.617.2 (Delta) variant then quickly spread. In North America, a larger variety of variants have been recorded. Globally, the Alpha variant was emerging as dominant until May 2021, when the Delta variant was introduced. The Delta variant has a global presence, with apparent rapid transmission once established in a region. The rapid global transition from the ancestral form of SARS-CoV-2 to the D614G (G clade) variant earlier in the pandemic more closely parallels the recent rapid transition to the Delta variant than the slower transition to the Alpha variant. The local regional transitions in sampling fractions also parallel these patterns.

Dr. Korber reported that the Alpha and Delta variants are cocirculating in Russia, India, and Australia. In India, the B.1.617.1 (Kappa) variant was the first to begin to increase in prevalence, then the Delta variant emerged and now is dominating global sampling. The B.1.351 (Beta) variant was first reported in South Africa and subsequently in Bangladesh, the Philippines, and Singapore, where the epidemics are much more complex. When the Beta variant had a significant presence, it persisted even when cocirculating with the Alpha variant. When the Delta variant was first reported in April and May in Singapore and Bangladesh, it rapidly became more frequently sampled. She noted that the P.1 (Gamma) variant, first reported in Brazil, is now very common in South America and it is increasing in frequency and gaining relative to the P.2 variant, which previously was identified globally, but it is declining rapidly. Dr. Korber reported that the Gamma variant is increasing compared to several other variants, including B.1.427/9, C.37, and Alpha variants. She noted that the C.37 variant emerged in Chile during spring 2021 during an aggressive vaccination effort and seems to be decreasing as Gamma is now predominant in Chile predominant, but that could change rapidly if the Delta variant has a similar trajectory of rapid expansion in South America as in other parts of the world. The C.37 variant contains multiple mutations, meriting further study. Additionally, more complex variants are preferentially arising.

Some of the most highly vaccinated populations are in the Seychelles, with 72 percent of the population vaccinated; Israel, with 63 percent of the population vaccinated; and Bahrain, with 60 percent of the population vaccinated. While confirmed SARS-CoV-2 cases are spiking in Seychelles and Bahrain, there have been no sequences from the Seychelles submitted to GISAID at the current time, and only 38 sequences from Bahrain have been submitted with none submitted more recently than April 2021. Dr. Korber suggested that this represents opportunities for partnership with clinical and sequencing groups in these areas. She noted that SARS-CoV-2 variants, including the C.37, Delta, and Alpha variants, emerging in Israel merit further study given the high vaccination rates in that nation. The C.37 and Alpha variants carry a 490S mutation which should be prioritized for testing against sera from vaccinated individuals.

Dr. Korber commented that all lineages of SARS-CoV-2 are dynamic and evolving into variants of variants. For example, in the full GISAID set of complete spike protein sequences, comprising >1,500,000 sequences, approximately 45% contain the common Alpha variant spike and additional mutations. She suggested that the field's priorities should be to determine the high frequency or fraction of variants among contemporary samples in various locales in the world, the repeatedly increasing fraction of new variants relative to the baseline Alpha variant and other VoCs regionally, and mutations in the NTD and RBD. The most common variant of the Alpha variant is B.1.1.7+K1191N. At the state and province level, it has an established presence in 53 regions. It is significantly increasing in nine regions—it currently is most common in Florida and significantly decreasing in 18 regions. A newer and rare, but interesting form is B.1.1.7+F490S, which has an established presence in 22 state- or province-level regions. It is increasing significantly in 14 regions—including Łódzkie, Poland, where it is most common and significantly decreasing in two regions. Many recurring mutations in the NTD and RBM are known to be associated with resistance, and the addition of a positive charge near the furin cleavage site is a highly recurrent pattern of mutation.

Dr. Korber provided an overview of the emerging lineage characteristics. Variants of interest generally carry one or more mutations in each epitope region of the RBD and NTDs, suggesting immunological selection in both regions. Variants of interest often carry likely or known infection-enhancing mutations. She commented that increasingly complex forms are replacing simpler forms, and indels in the NTD of the Spike protein are becoming much more extensive and common. Repeated mutational patterns suggesting convergence is very common, and these patterns are concentrated in the RBD and NTDs, and the furin cleavage site. Recombination is occurring among these viruses. Dr. Korber emphasized that much remains unknown about these variants, and additional resources and partnerships are urgently needed with clinical and sequencing groups in South America, Africa, and Asia.

Dr. Korber underscored that these mutations and variants of SARS-CoV-2 have implications for vaccine design. Current evidence suggests that this virus may be evolving in sweeps. The G clade evolved to the Alpha variant, which evolved to the Delta variant. Transitions to global prevalence can occur quickly; the transition to the G clade in the spring of 2020 took 6 to 10 weeks, and it is possible this pace of transition may be happening for the Delta variant. She noted that although the exact trajectory of future variants cannot be predicted, patterns of convergence and covariation can be studied to determine which relevant variants and forms. This information will inform the design and development of a polyvalent COVID-19 vaccine candidate(s) and translation to new COVID-19 vaccine platforms.

Session I: Mechanisms of Action of Functional Antibodies That Neutralize SARS-CoV-2 and/or Eliminate SARS-CoV-2 Infected Cells

Presenter: Erica O. Saphire, Ph.D., La Jolla Institute for Immunology

Dr. Saphire presented on collaborating to accelerate the search for therapeutic antibody cocktails against SARS-CoV-2. Therapeutic antibody cocktails to treat viral infections like SARS-CoV-2 also require careful selection and mixing to optimize their protective efficacy.

The Continued Threat of SARS-CoV-2

Since its emergence in late 2019, SARS-CoV-2, the causative agent of COVID-19, has disrupted lives and livelihoods around the globe. After several substantial surges of infections, by June 2021 SARS-CoV-2 has infected over 175 million people and resulted in over 3.8 million deaths.[13] Two mRNA-based vaccines [BNT162b2 from Pfizer/BioNTech,[14] mRNA-1273 from Moderna[15]] and one vaccine using an adenovirus platform Johnson & Johnson Ad26.COV2.S[16] have received FDA Emergency Use Authorization (EUA) (Oxford–AstraZeneca AZD1222[17] is approved for use in other countries). Despite the unprecedented pace of vaccine development, vaccine supply and uptake remain a challenge. By early June 2021, around six months after the vaccines were first granted EUA, only 12% of the world's population has received one dose of any vaccine, and the majority of these people live in developed nations[13] and in some of these developed countries, vaccine rates have plateaued. The vast majority of unvaccinated people are in low- and middle-income countries, where, in many places, social distancing is impossible. Until the majority of the world's population is vaccinated against SARS-CoV-2, this virus will continue to be a threat and those infected with SARS-CoV-2–especially those who have risk factors associated with severe disease–will need access to effective therapeutics.

SARS-CoV-2 Spike: The Target of Immune Response

SARS-CoV-2 infection of host cells is mediated by the spike glycoprotein that exists as a trimeric glycoprotein.[18, 19] Each spike monomer has two domains, S1, which includes the N-terminal domain (NTD) and receptor binding domain (RBD), and S2, which includes the fusion domain that governs fusion between host and virus membranes. S1 and S2 are separated by a furin cleavage site.[20] SARS-CoV-2 interacts with angiotensin converting enzyme 2 (ACE2) as its main receptor to gain cell entry.[19, 21] Following receptor binding, the S1 subunit is cleaved by the plasma membrane-associated serine protease, TMPRSS2.[22] This cleavage in turn promotes transition from the pre-fusion to a fusion-competent form that drives membrane fusion and allows entry into target cells. As the major feature on the virus surface, the spike protein is the prime target of antibody responses.[23]

Therapeutic Monoclonal Antibodies to Treat SARS-CoV-2 Infection

Two monoclonal antibody (mAb) cocktails and one mAb monotherapy have received EUA to treat individuals infected with SARS-CoV-2 who are not yet hospitalized but are at risk of developing severe disease, or for high-risk individuals have been exposed to an infected person.[24] There are more than a dozen other candidate therapeutics that are at various phases of clinical testing.[25, 26] Regeneron's REGN-COV2 two antibody cocktail includes one antibody isolated from a convalescent COVID-19 patient and one isolated from genetically humanized mice immunized with spike protein.[27] Two antibodies, LY3819253 [LY-CoV555; Bamlanivimab[28]] and LY-CoV016 [LY3832479, etesevimab[29]] were isolated from plasma B cells of convalescent COVID-19 patients within one month of symptom onset, as part of a collaboration of Eli Lilly, AbCellera and the NIH. In late May

2021, Vir Biotechnologies received EUA for their single mAb therapeutic, VIR-7831 (sotrovimab), which was isolated from memory B cells from a survivor of the 2003 SARS outbreak.[30] All of these antibodies target the spike RBD. These antibodies are being deployed to treat patients infected with SARS-CoV-2, but, as with the vaccine, many individuals, particularly those in low- and middle-income countries, lack access.

The Coronavirus Immunotherapy Consortium (CoVIC): A Collaboration to Identify Novel Therapeutic Antibody Cocktails

The Coronavirus Immunotherapy Consortium (CoVIC)[31, 32] launched just two months after the initial sequence of the SARS-CoV-2 virus was reported.[33] This international effort is headquartered in the Saphire lab at the La Jolla Institute for Immunology (LJI) and has two main goals. A translational research goal is to identify highly potent monoclonal antibody cocktails against SARS-CoV-2 spike protein that can be used to save lives of individuals living in low- and middle-income countries who are infected with SARS-CoV-2 and at high risk of developing severe COVID-19. A fundamental research goal is to provide a broad and deep database of antibody activities against SARS-CoV-2 spike protein, evaluate therapeutic candidates side-by-side under standardized platforms, and use this broader array of mAbs and deep body of information to make ideal combinations. A long-term goal is to develop a profile of *in vitro* antibody activity that best correlates with protective clinical efficacy, as well as assess the ability of *in vitro* assays and *in vivo* models to predict clinical success.

To achieve these parallel goals, the CoVIC is compiling a panel of antibodies that in one year has grown to number over 350 candidate antibody therapeutics. The panel is unique in that it comprises therapeutic candidates from over 50 groups across four continents working in industry/biotech, academic, government, or independent research institutes. The contributions from industry came from companies ranging from those with fewer than a dozen employees to multinational corporations with thousands of employees. Importantly, the CoVIC panel is anonymized through assignment of a code numbers that allows each mAb in the CoVIC panel–regardless of whether it came from the smallest company or the largest, from academia or from industry–to be evaluated on an equal footing with all the others. Moreover, the CoVIC panel includes antibodies that were isolated from COVID-19 survivors, phage display, naive libraries, in silico methods or other strategies. Each was elicited and evaluated using distinct criteria. Together with the relatively unrestricted inclusion criteria (<100 nM affinity for spike), the CoVIC panel represents a broader, deeper array of antibodies than other studies that may have considered antibodies derived from only a few patients, or those isolated using one approach.

A Battery of Assays

All the items in the CoVIC panel are being tested side-by-side, in apples-to-apples comparisons, in a battery of standardized assays performed by 8 different partner labs (Figure 1). Binding studies are performed in a Good Laboratory Practice (GLP) setting at Dr. Georgia Tomaras' laboratory at the Duke Human Vaccine Center and at Carterra, both using high-throughput surface plasmon resonance (SPR) in the Carterra LSA platform. Binding studies will assess affinity of CoVIC antibodies for a range of spike antigens (full-length ectodomain, soluble RBD, NTD and spikes bearing mutations from VoCs (see below). Importantly, all antigens are structural biology-grade with high purity and uniformity. The Saphire lab (LJI) performs cryoEM and X-ray crystallography to map epitopes, binding angles, and binding of IgG vs. Fab. Three sets of neutralization data, two involving authentic virus (Baric group at University of North Carolina and the Bukreyev group at University of Texas Medical Branch Galveston) and one involving pseudovirus on a rVSV backbone (Nexelis) will be generated for each CoVIC item. Propensity and location of escape mutations are being mapped by Yoshihiro Kawaoka (Wisconsin).

The Alter group at the Ragon Institute are examining Fc-mediated activity in terms of ability to inspire phagocytic activity or natural killer (NK) activation, as well as affinity for a range of Fc receptors (FcRs). *In vitro* protection is analyzed by Jordi Torelles and Luis Martinez at Texas Biomedical Research Institute using mouse (transgenic for human ACE2 under the control of the K18 promoter) and hamster models as well as at LJI by Sujan Shresta using a huFcRN mouse model.

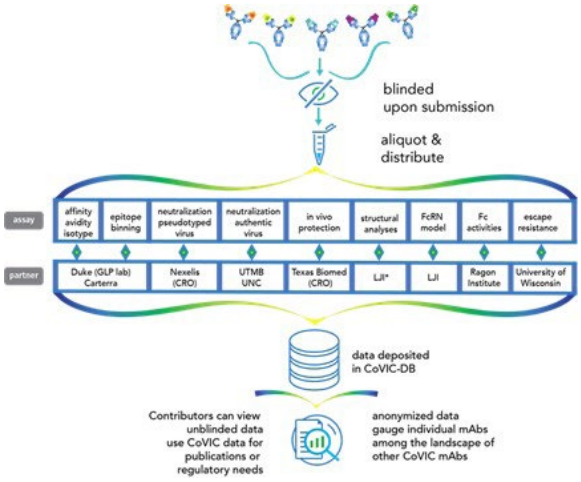


Figure 1. *Organization of the CoVIC. Antibodies sent by contributors arrive at LJI where they assigned code names and aliquoted for dispatch to the eight partner labs. After performing their analyses, the partner labs upload the data into the CoVIC database (COVIC-DB).*

CoVIC Database "CoVIC-DB"

To facilitate FAIR (findable, accessible, interoperable, reusable) analysis,[34] all comparative data generated by the CoVIC partner reference labs are uploaded into a publicly accessible database (covic.lji.org/databases), the CoVIC-DB, which is being developed by Bjoern Peters' group at LJI. The CoVIC-DB will carry results for a standardized set of neutralizing antibodies that were produced at scale by LakePharma and shared with partner reference labs. Analysis of data for anonymized antibody samples in the COVIC-DB will expand understanding of the landscape of antibody activities against SARS-CoV-2, including how epitope footprints correlate with different functions, which Fc functions predominate at which epitopes sites, which characteristics lead to most potent neutralization, and which potential escape mutations impair which classes of antibodies. Antibody contributors will know which samples in the database are theirs and can contact the Program Manager, Dr. Sharon Schendel, should a particular datapoint not match expectations. The CoVIC-DB now carries data for the first 270 antibodies in the panel (other samples are under analysis). These data can be viewed in an interactive format or may be downloaded as a .csv file for analysis in Excel, Prism, or other software.

Adaptability of CoVIC to the Search for Durable Antibody Therapeutics Against SARS-CoV-2 VoCs

Outside of the emergence of the D614G variant early in the pandemic, throughout the first 11 months of the COVID-19 pandemic, the amino acid sequence of SARS-CoV-2 remained relatively static until the B.1.1.298 variant was first observed in June 2020 among several workers at a mink farm in Denmark.[35, 36] Although this variant did not become widespread, it did demonstrate the ability of SARS-CoV-2 to transmit from animals to humans and vice versa, as well as the potential to mutate in an animal reservoir. It was perhaps a harbinger of subsequent VoCs that would soon emerge.

VoCs refer to SARS-CoV-2 lineages that are associated with increased transmissibility and typically carry multiple convergent mutations that may have arisen either during chronic infection or in previously infected individuals.[37-42] In September 2021, the B.1.1.7 (Alpha) VoC,[43] which was first identified in the United Kingdom, emerged, and was quickly followed by several other VoCs, notably B.1.351[44] (Beta; first identified in South Africa) and P.1[45] (Gamma; first identified in Brazil). India first identified infections with B.1.617[46] (Delta). All are associated with enhanced human-to-human transmission. Among the array of mutations present in VoCs, many occur at the same sites and

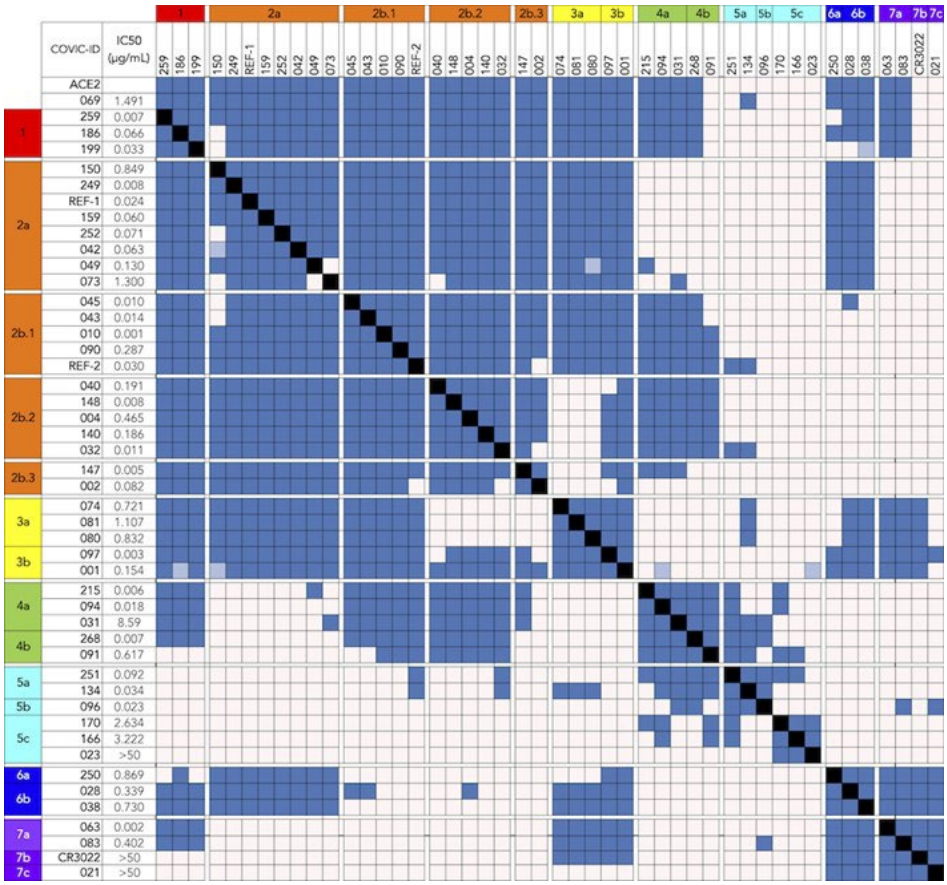


Figure 2. *Binary heat map for RBD-directed antibodies. The competition matrix shows pairwise competition results from a classical sandwich epitope binning with monomeric RBD using HT-SPR. Rows indicate immobilized antibodies and columns represent injected analyte antibodies. Blue and cream cells indicate blocking and sandwiching pairs, respectively. Light blue shading represents ambiguous classification. Black shading indicates self vs. self. The heat map was sorted to cluster like groups using Carterra Epitope software. IC50 values for neutralization of G614 pseudovirus are shown for reference.*

are evidence of likely convergent evolution. CoVIC had to rapidly pivot to analyze the ability of the antibodies in the consortium to retain neutralization to emerging mutations and VoCs. Here, we describe footprint analysis, structural biology, and neutralization that together organize the mAbs of the CoVIC panel into a framework from which mutation-resistant, therapeutic cocktails can be derived.

Several mutations in VoCs occur in the spike RBD, and in particular the receptor binding motif (RBM). The RBD is mobile and tends to oscillate between a “closed”, or “down” state, and an “open” or “up” state. RBM mutations found at K417, L452, E484 and N501 all appear to alter the interaction of spike with ACE-2[47] and in turn can impact interactions of antibodies that target the RBM and RBD. Mutations at N439 and S477 are also observed independently in patient samples.[48-50] Meanwhile, other mutations are associated with cross-species transmission (e.g., V367F, Y453F, and F486L). The N-terminal domain (NTD) is particularly vulnerable to sequence deletions that can affect the increasing number of antibodies that are known to bind this region.

To characterize the landscape of epitopes targeted by antibodies in the CoVIC and how antibody binding and neutralization could be affected by emerging VoCs, we first undertook a large-scale, high-resolution epitope binning of the first 270 antibodies in the CoVIC panel using the Carterra LSA platform and soluble RBD as the antigen to sort the landscape of epitopes targeted by antibodies in the panel into competition groups or “communities” (Figure 2).

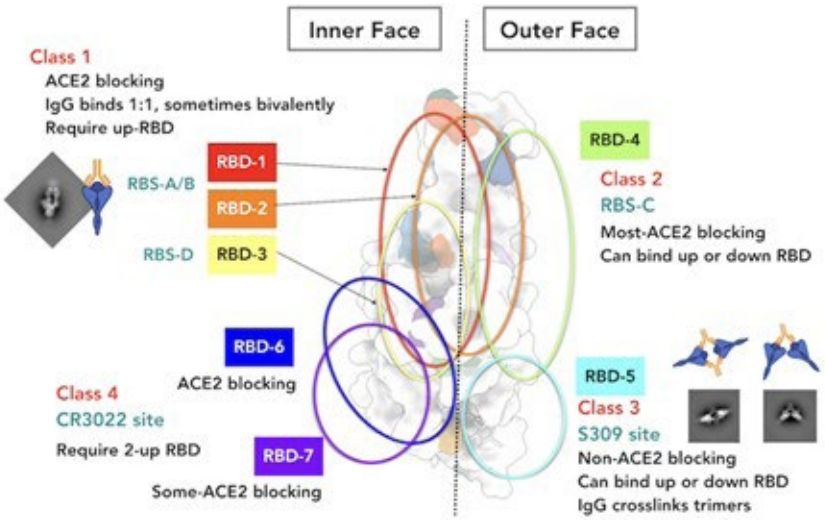


Figure 3. *Summary of binding patterns of the RBD communities. Antibodies in different communities approach the spike RBD from different directions. Notably, the representative nsEM for RBD-5 suggests that these antibodies can bind multiple spike trimers simultaneously.*

We next measured the affinity of the mAbs for G614-HexaPro spike[51] trimers and monomeric RBD, as well as blockage of ACE2-spike interactions. The majority of the antibodies in the CoVIC are targeted to the RBD, which was the focus of early antibody discovery efforts, although there are several that target the NTD or S2 domain. To visualize epitope footprints, we used negative stain electron microscopy (ns-EM) to determine low resolution structures of antibody Fabs, or, in some cases, intact IgGs, in complex with full-length HexaPro spike for at least two representatives of each community. At LJI, we also measured the neutralization activity of 43 RBD-directed mAbs (~4 members from each group) against VSV-based pseudovirus bearing a range of mutations–either singly or together–to reflect the sequence changes seen in VoCs. These 43 representative antibodies were chosen agnostically for breadth across the communities and sub-community branchpoints not according to the germline origin, CDR features or length or antibody origin. This group includes antibodies from a range of origins (e.g., human, mouse, in silico) and formats (e.g., IgG, scFv, VHH, multivalent).

From results of high-throughput surface plasmon resonance (HT-SPR), we produced a competition profile that allowed us to distinguish 189 RBD-reactive mAbs. These RBD-directed antibodies were sorted into 7 core "communities" based on the competition profiles of each mAb to the others. Note that the first branch point separates two of the seven from the other five. The next branch point subdivides the five into a group of three and a group of two. Communities RBD-2-RBD-7 can be further subdivided into clusters and more traditional epitope bins by considering how they compete with antibodies in other clusters and with ACE2 for spike interactions. We next performed nsEM on at least two members of each group and several major subdivisions to understand the physical footprints of each of these competition groups. The first three branch points divide antibodies into those against the receptor binding motif (RBM; groups RBD-1, RBD-2, and RBD-3), the inner face (exposed in the up position; RBD-6 and RBD-7) and the outer face (exposed in up or down; RBD-6 and RBD-7). The subdivisions in the competition analysis map to distinct shifts in positioning within each major footprint (Figure 3, 4).

RBD-1, together with RBD-2 and -3, correspond to "Class 1" antibodies described by Barnes et al.[52] REGN10933 is the canonical member of Class 1. In the broad CoVIC analysis, the RBD-1 group contains ACE2 itself and antibodies with epitopes that more directly overlap with that of ACE2 (Figure 2). Antibodies in RBD-2 are shifted slightly toward the outer face from RBD-1. Those in RBD-3 have footprints shifted toward N501. RBD-4 and RBD-5 antibodies approach spike from

the outer face of the RBD, and roughly correspond to Class 2 [e.g., C002[53]] and Class 3 (e.g., REGN 10987), respectively. Meanwhile, RBD-6 and -7 attack spike from the inner face of the RBD and are similar to Class 4 antibodies like the SARS-derived CR3022.[54] Our subdivision of class 4 into the distinct groups RBD-6 and RBD-7 is based on differences in competition. RBD-6 competes with the potent neutralizers of RBD-2, while RBD-7 does not. RBD-6 also has greater propensity to block ACE2.

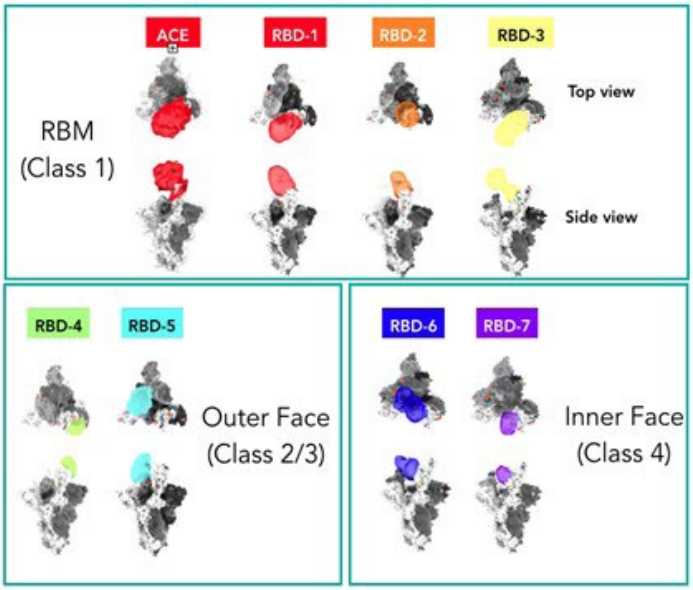


Figure 4. *Binding footprints of representative antibodies from each of the seven RBD communities. Spike trimers are shown with monomers colored white, gray and black. Bound Fab fragments determined by NS-EM are shown according to the color scheme shown in* Figure 2. *Top and side views are shown.*

RBD-2, for example, has "a" and "b" sub-clusters that contain mAbs that do and do not compete, respectively, with mAbs in RBD-4. The larger RBD-2b cluster can be subdivided into three groups. The mAbs in 2b.1 can compete with RBD-3 mAbs, but 2b.2 and 2b.3 do not. Meanwhile, mAbs in 2b.1 and 2b.2, but not those in 2b.3, compete with those in RBD-4. The RBD-4 class itself can be subdivided into two groups, 4a and 4b that do and do not, respectively, compete with ACE2.

The clustering helps define susceptibility to mutations of concern. The potency of RBD-2a antibodies is diminished by the K417N mutation, but less so by the E484K mutations. RBD-2b has the opposite profile: affected by E484K, but more resistant to K417N. On the other hand, RBD-3 is affected by both the E484K and N501T/Y mutations. The RBD-1 mAbs vary in their susceptibility to particular mutations that may be related to nuances in the ACE2-centered footprint. Nearly all members of RBD-1 and -2 show additive decreases in neutralization potency of pseudovirus carrying mutations present in prominent VoCs. Despite being relatively impervious to K417N, E484K and N501Y alone, the IC50 for several of these highly potent mAbs falls by up to 1000-fold against B.1.355 and P.1 (Figure 5). Most RBD-1 and -2 mAbs are unaffected by B.1.1.7, which has fewer RBD mutations. This finding is consistent with that for bamlanivimab and imdevimab that retain full neutralization activity against B.1.1.7.[55]

Communities RBD-4 and -5, like Class 2 and Class3 mAbs, bind RBD either in the "up" or "down" conformation. Although their footprints overlap, the RBD-4 mAbs target the outer edge of the RBM and block ACE2 interactions, while the binding site for RBD-5 is away from the RBM site, which, like the previously described S309, renders it unable to block ACE2.[47] The E484K mutation affects most RBD-4 antibodies, as does the N439K mutation. Meanwhile, neutralization by RBD-5 antibodies is broadly resistant to nearly all mutations tested (Figure 5).

RBD-6 and RBD-7 target the inner face of the RBD to hit a previously described cryptic epitope.[56-58] These antibodies prefer RBDs in the "up" configuration. They block ACE2 binding, except for those in the subclusters RBD-7b and RBD-7c. The neutralization activity of representatives from these two groups was poor, but they are resistant to the effects of VoC mutations. Like RBD-4 and -5, the

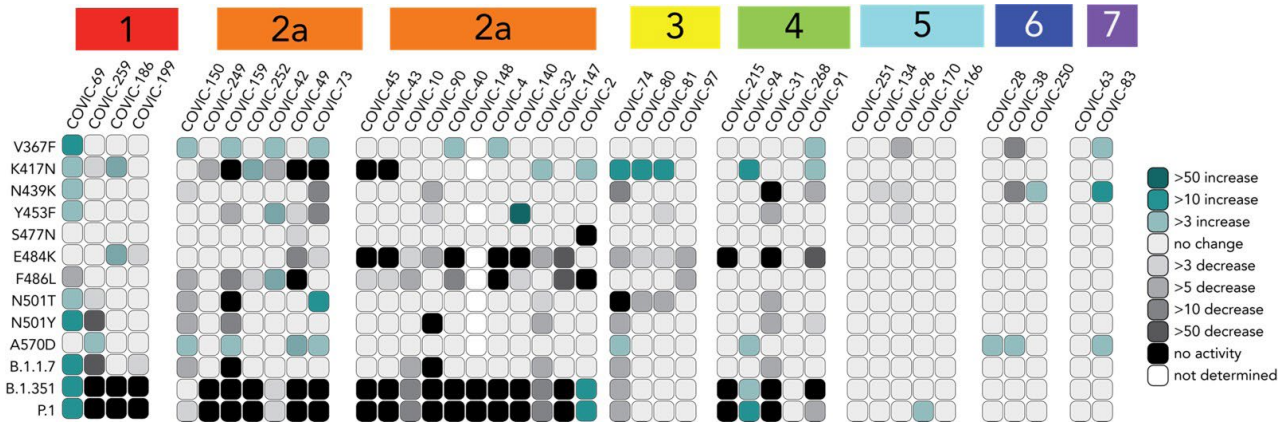


Figure 5. *Neutralization activity against pseudovirus bearing individual point mutations or the full complement of mutations found in VoCs. Of note, mAbs in RBD-5 are largely resistant to all prominent circulating mutations.*

footprints of RBD-6 and -7 are similar, but RBD-7 antibodies are able to bind spike with those in RBD-2a due to the downward shift of the epitope along the RBD inner face relative to that for RBD-6.

Antibodies against the NTD bind in a continuum of sites. Three example NTD-directed antibodies shown here, belonging to groups we term NTD-1, NTD-2 and NTD-3, which together cover the extremities of the NTD supersite. NTD-1 binds from the top side to contact Y144 and overlaps with the footprint of mAb 4A8[59] as well as other characterized supersite binders. NTD-2 antibodies approach NTD from the front and also contact Y144 in addition to H69, V70 and G261. The NTD-2 footprint mirrors that for the antigenic site V group described by McCallum et al.,[60] while the NTD-3 mAb binds to the inner side of the NTD. The neutralization activity of all NTD-targeted antibodies analyzed was either diminished or totally lost against pseudovirus bearing deletion mutations (Δ69/70, ΔY144 and Δ242-244), which are prominent in circulating VoCs–particularly B.1.1.7. In general, NTD-directed antibodies are susceptible to the frequent mutation and deletion in the NTD in the VoCs.

One NTD-1 antibody, COVIC-246, however, shows the greatest resistance to NTD mutations. It retains neutralization activity against pseudovirus carrying the full complement of mutations present in B.1.351, but, interestingly, lost activity toward pseudovirus that had the 242-244 deletion alone. This result suggests that some mutations in VoCs could have offsetting, compensatory effects that allow retention of neutralization activity. Our and others' results indicate that antibodies targeting NTD might be susceptible to mutations in VoCs, but given the potential for offsetting effects, may still be valuable members of therapeutic cocktails.

Taken together, of the 43 RBD-directed mAbs tested, those in communities RBD-5, -6 and -7 maintained nearly complete neutralization of pseudoviruses carrying either point mutations or the full complement of mutations in emerging SARS-CoV-2 variants. The epitopes targeted by antibodies in these communities are conserved among the Betacoronavirus subgenus B that includes SARS and SARS-CoV-2.[61] In RBD-5, which contains antibodies that can bind RBD in either the "up" or "down" conformation,

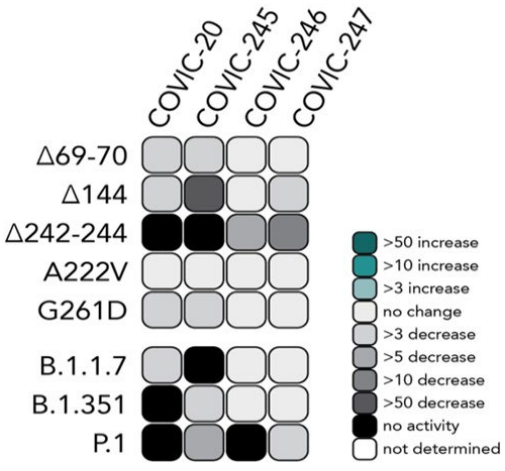


Figure 6. *Neutralization profile of NTD-targeted mAbs against pseudovirus bearing individual point mutations or the full complement of mutations present in circulating VoCs.*

nsEM of intact IgG for one antibody was seen to crosslink spike trimers, suggesting that it may have a novel mechanism of action that could be tied to the angle of attack for these antibodies. Some outliers include COVIC-2, an RBD-2 mAb which, in contrast to other members of that community, actually gains neutralization potency against B.1.351 and P.1, as well as COVIC-246, the NTD-antibody that resisted common deletions and point mutations seen in VoCs.

Overall, antibodies from community RBD-1 through RBD-4 and those directed against the NTD are generally more potent than mAbs of other communities. The high potency and non-overlapping epitopes of RBD- and NTD-directed mAbs make them attractive as pairs for therapeutic cocktails. However, members of each of these groups are also highly susceptible to neutralization escape by mutations and deletions found in emerging VoCs. A bi-specific antibody targeting the RBD-1 and NTD-1 sites can still neutralize single point mutations in the RBD (where the NTD arm of the bispecific could compensate), but is ineffective against the B.1.351 and P.1 variants, which contain mutations that escape both arms of the bispecific at once. We note, however, that the RBD-2 community and other antibodies in NTD-1 contain mAbs (CoVIC-2 and CoVIC-246, respectively, as examples) that are largely resistant to deletions and point mutations present in VoCs. Hence, careful selection of mAbs from these groups may facilitate a potent and variant-resistant cocktail.

In contrast, RBD-5, -6 and -7 mAbs often have lower potency but are more resistant to escape. Notably, the epitopes targeted by RBD-5, -6, and -7 mAbs have high sequence conservation among the *Sarbecovirus* subgenus of *Betacoronavirus*. Enhanced potency for these communities might be achieved through engineering them for potency or as multivalent formats. These RBD-5, -6 and -7 antibodies could be key members of a variant-resistant cocktail that could also be suitable for treating other *Sarbecovirus* infections.

This broad analysis using one of the largest, and most varied collection of anti-spike antibodies yet compiled, shows that determination of binding modes can provide a foundation for understanding how antibodies target spike and how spike mutations facilitate escape. However, nuances in activity among these communities show that there is no one-size-fits all approach to identifying therapeutic candidates. Additional analysis of Fc effector functions and performance in animal models and clinical studies will further guide treatment selection. Careful stirring will be required to develop antibody cocktails that have the breadth and potency necessary to fulfill cost of goods requirements and ensure that all have equitable access to life-saving treatments for SARS-CoV-2 infections.

CoVIC Methods

Binding: Binding studies are performed in a Good Laboratory Practice (GLP) setting, at Dr. Georgia Tomaras' laboratory at The Duke Human Vaccine Center. High-throughput surface plasmon resonance (SPR) using the Carterra LSA platform and biolayer interferometry (BLI) assays using the Forte Octet will assess binding kinetics to full-length spike, D614G and prominent VoCs. The association and dissociation rates for the receptor-binding domain (RBD) and N-terminal domain (NTD) will also be determined, as will the ability of mAbs to block ACE2 binding. High-resolution epitope binning of the range of therapeutic candidates will be performed.

Neutralization: Neutralization capacity of mAbs contributed to the CoVIC will be evaluated using a pseudovirus system and two assays involving authentic virus (both D614G and VoCs). Pseudovirus neutralization assays are performed by Nexelis using a method described by Whitt et al.[62] that uses genetically modified Vesicular Stomatitis Virus from which the glycoprotein G is removed (VSVΔG). VSVΔG virus is transduced in HEK293T cells previously transfected with SARS-CoV-2 coronavirus spike (Wuhan strain, accession NC_045512) lacking the last 19 amino acids of the cytoplasmic tail

(ΔCT). The resulting pseudoparticles (VSVΔG-Spike ΔCT) contain a luciferase reporter to provide a signal that can be quantified in relative luminescence units (RLU). Neutralization activity is assessed using 11-point concentration curves (mAb concentrations ranging from 0.004-3.6 µg/mL) from which IC50 and IC90 values will be determined from four-parameter logistic curves.

Neutralization of authentic SARS-CoV-2 will be performed by the Bukreyev group at the University of Texas Medical Branch, Galveston (UTMB) and by the Baric group at University of North Carolina (UNC). At UTMB, neutralization is assessed using virus engineered to express mNeonGreen (SARS-CoV-2-mNG)[63] for high-throughput measurement and a readout that is more consistent than that achieved with plaque reduction neutralization tests (PRNTs) and manual counting. Under BSL-3 containment, mAbs at a range of concentrations will be incubated with SARS-CoV-2-mNG for one hour prior to application to Vero E6 cells pre-seeded in 96-well plates at a MOI=0.5. At 16 h post-infection, mNeonGreen-positive cells indicating infection will be quantified using a high-throughput imaging reader.[64] Neutralization curves will be generated, from which IC50 and IC90 values will be determined. Neutralization activities measured by PRNT and with high-throughput SARS-CoV-2-mNG microneutralization assay were shown to be comparable (R2=0.90). The UNC assay is performed as described by Hou et al.[65] Briefly, under BSL-3 containment, serially diluted mAbs at 8 concentrations are incubated for one hour with 87 PFU/well D614G-nLuc virus for one hour at 5% CO_2 and 37 ˚C. After incubation, the virus/antibody mixtures are added in duplicate to black-walled 96-well plates containing Vero E6 cells (2 x 10^4 cells/well). Each plate contains virus-only control wells. The plates are incubated for 48 hr at 37 ˚C, 5% CO_2 and the cells are lysed before measurement of luciferase activity with the Nano-Glo Luciferase Assay System (Promega) according to the manufacturer's instructions. Neutralization activity is expressed as the concentration at which the observed relative light units (RLU) are reduced by 50% relative to virus-only control wells.

Structural biology: Cryo-electron microscopy (cryo-EM), cryo-electron tomography (cryo-ET) or X-ray crystallography using recombinant spike, transmembrane-anchored spike, or RBD or NTD domains, respectively, will be performed by the Saphire laboratory at LJI using two dedicated 300 keV Titan electron microscopes with direct electron detectors and a Gatan energy filter, as well as an Aquilos instrument and cryo-correlative light and electron microscopy (cryo-CLEM) for visualization of structures in their biological, transmembrane context. Footprints, contact residues, stoichiometry, binding angle, adjustments to spike and synergy upon binding can be visualized with high-resolution structural biology. Our laboratory routinely does both cryoEM and X-ray for single particle structural analysis, with cryo-EM used to visualize complexes with trimeric spike and X-ray used to visualize complexes with RBD or NTD.

Escape and Surveillance: Resistance to mutagenic escape and maintenance of neutralization against VoCs is critical for durability of candidate therapeutics. Bette Korber's group (Los Alamos National Laboratory) is surveying the GISAID database of coronavirus sequences to guide experimental evaluation of the ability of candidate therapeutics to remain responsive to different variants.[66] Specific escape mutations will be identified by Yoshihiro Kawaoka's laboratory at the University of Wisconsin using rVSV and authentic SARS-CoV-2 with Vero E6 cells engineered to express TMPRSS2. Single mAbs and mAb combinations will be analyzed for escape propensity and location of escape mutations in the spike protein. mAbs will further be screened for binding and/or neutralization of other escape mutants. The goal is to identify a series of clinical candidates having differing susceptibilities, so that all candidates in use are not escaped by the same mutation and a library of antibody therapeutics can be maintained for use in multiple seasons.

Fc profiling, systems serology: The Alter Laboratory at the Ragon Institute of MGH, Harvard and MIT will profile the ability of each therapeutic antibody candidate to inspire a range of Fc-mediated activities. Functions profiled will include antibody-dependent cellular cytotoxicity, phagocytosis, activation and maturation of innate immune effector cells, cellular degradation, complement deposition, and antigen uptake of innate immune cells. Cells surveyed will include NK and dendritic cells, neutrophils, monocytes and macrophages, and both human and murine effector cells[67-69] for comprehensive capture of cross-species correlates with immunity against SARS-CoV-2.[70-72] These studies will capture multiple data points for each mAb, and will be linked to structural and *in vivo* findings, using both univariate and multivariate tools, to define relationships between antibody effector profiles and epitope specificity and Fc features that track with protection against SARS-CoV-2.

Fc profiling, cellular studies: The Burkreyev lab will evaluate whether CoVIC mAbs can induce innate immune effector function in a range of cellular assays using primary human myeloid cell populations (monocytes, macrophages, immature and mature dendritic cells (DCs) and NK cells) isolated from human donor blood by magnetic sorting.[73, 74] They will also evaluate the dependence of enhanced uptake on avidity of Fc domain-Fc receptor interactions, the effects of blocking each main type of Fcγ receptor on enhanced uptake,[74] and characterize the role of specific Fc effector functions.[75] Any links between isotype, epitope, neutralization capacity or other characteristics and enhanced uptake *in vitro* are also being established.

***In vivo* analysis:** Texas Biomedical Research Institute, under the leadership of Dr. Jordi Torrelles, is evaluating the efficacy of CoVIC mAbs beginning with a mouse model of disease involving expressing human ACE2 under control of the K18 promoter.[76] CoVIC mAbs are delivered prophylactically to 4 - 6 week-old mice (males and females) at a minimum dose of 0.5 mg/kg one day prior to infection via intranasal inoculation of $2x10^5$ PFU/animal SARS-CoV-2. Morbidity (weight loss) and mortality will be monitored up to 10 days post-infection (dpi) and nasal turbinates and lungs will be collected on 2 and 4 dpi for pathological evaluation at Nexelis. Those antibodies that show protective activity will also be tested in a therapeutic model in which the antibody is delivered after virus infection. A hamster model is also being developed and will be used to test antibodies that perform well in the mouse model.

In a separate *in vivo* study, Sujan Shresta's group at LJI is evaluating the *in vivo* performance of some CoVIC mAbs using a novel triple knockin mouse model that is transgenic for human ACE2, human FcRn and human TMPRSS2. As an animal model of COVID-19, these mice could better recapitulate mAb pharmacokinetics (PK), and potential risk factors for antibody-dependent disease enhancement (ADE). The clinical success of a therapeutic CoVIC mAb can be tied to longevity (half-life), a feature related to its affinity for the FcRn receptor, which is expressed on endothelial cell membranes and constantly endocytoses IgG from the plasma and recycles it back into the plasma. The 10-fold higher affinity of mouse FcRn for human IgG compared to human extends the half-life of human mAbs, which could result in poor recapitulation of in mice. Thus, mouse models provide comparatively poor representation of human pharmacokinetics[77-81] and can complicate modeling of Ab half-lives, virus neutralization, and likelihood of therapeutic Ab-FcRn interactions and immune complex formation. mAbs engineered to have minimal Fc receptor binding (e.g., LALA mutant antibodies) have normal human FcRn binding sites and interactions.[82] whereas Abs engineered to have extended half-life (e.g., YTE, LS, Xencor Xtend mutations) have enhanced FcRn affinity. FcRn may also play a role in endothelial cell uptake of immune-complexed SARS-CoV-2 virions and could promote presentation of viral antigens to CD4 and CD8 T cells to bridge humoral and cellular immunity.[83, 84]

Discussion Panel

Moderator: Adrian McDermott, M.Sc., Ph.D., NIAID, NIH

Panelists: Christopher Barnes, Ph.D., Stanford University; Michael Diamond, M.D., Ph.D., Washington University in St. Louis; David Montefiori, Ph.D., Duke University; Penny Moore, Ph.D., University of the Witwatersrand, Johannesburg; Rachel Liberatore, Ph.D., RenBio, Inc.; and Laura Walker, Ph.D., ADIMAB, LLC

Dr. McDermott led the Panel discussion on the prospect of combination therapies. Dr. Christopher Barnes emphasized the importance of focusing on VoCs, particularly on the location of the mutations in the spike protein and identifying which classes of antibodies can be combined to address commonly occurring escape mutants. He suggested that combining antibodies from different classes or groups could improve efficacy. Dr. Barnes also proposed that combining two RBD binders with one NTD binder would provide more coverage on the spike protein and allow antibody cocktails that are more potent. He cautioned that structural considerations must be understood fully to ensure that antibodies with different orientations (angles of approach) do not block each other.

Dr. Rachel Liberatore commented that the focus on conserved epitopes for antibody therapeutics is important for addressing variants. She noted that the orientation and shape must be considered, especially for bi-specific antibodies. Dr. Laura Walker explained that ADIMAB, LLC is focusing on broadly neutralizing antibodies (bNAbs), which could be a long-term solution. Some Class 3 antibodies that currently are resistant to escape may not be so in the future, therefore new cocktails may be needed. She noted that bNAb target sites are not usually targeted by other antibodies. When asked about the challenge of variants, Dr. Walker responded that her team has not identified variants resistant to their bNAbs, but some variants have mutations that allow them to escape specific antibodies. She proposed that a cocktail of two bNAbs could provide increased protection, but variant trajectories are difficult to predict.

Dr. Michael Diamond commented that one key component to down-selection of antibody treatment options is identifying antibodies that can bind at conserved sites and are highly potent. Although use of mAbs does not appear to result in viral escape to occur easily, the use of a cocktail with a second antibody also would increase control over viral resistance, which typically does not happen under conditions of antibody drug combinations. He commented that small animal models have shown the importance of antibody effector function, but translation of this function from animals to humans is not always predictable. Dr. McDermott asked the Panel members whether the difference in protection observed between *in vitro* and animal models indicates Fc function. Dr. David Montefiori confirmed the importance of Fc effector function. Dr. Penny Moore added that her laboratory studies this in the context of infection. She noted that there is a general lack of understanding about the targets of the most potent Fc effector function antibodies, which may not overlap with the antibodies most researchers currently focus on isolating. Dr. Diamond commented that Fc effector function needs to be categorized based on antibody activity—not all antibodies bind to the spike protein equivalently, and this may correlate with protection. He commented that some animal models have shown the therapeutic activity of NAbs is augmented substantially by Fc effector function. This question provides the opportunity to gain more information on how antibodies engage the spike protein and optimize those functions to enhance antiviral activity over time. When asked whether antibodies that bind to the S1 subunit and cause the spike trimer to "shed" preventing the virus to infect another cell, Dr. Diamond commented that transduced and viral S subunits show differences, and validation is required. Dr. Walker commented that many *in vitro* assays are performed with stabilized S protein.

Dr. McDermott asked Panel members about the potential to improve antibody efficacy. Dr. Walker noted that bNAbs from humans often have a tradeoff between breadth and potency. She commented that her team is engineering antibodies to overcome this challenge. Dr. Diamond commented that his team has found an effective antibody in mice and agreed that engineering could provide an antibody that offers both breadth and potency in humans. Dr. Liberatore commented on the enhanced potency of bi-specific antibodies. Dr. Barnes noted that his team is exploring libraries of antibodies to identify opportunities for improvement as many human antibodies have high affinities. Dr. Barnes plans future studies to determine whether antibodies resulting from COVID-19 vaccination are effective against SARS-CoV-2 variants.

Session II: Preclinical Delivery, Pharmacology, and Efficacy of Anti-SARS-CoV-2 Antibodies

Presenter: Ralph Baric, Ph.D., The University of North Carolina at Chapel Hill

Introduction

- Some animal models show good correlations between direct acting antivirals in small animal performance *in vivo* and protection in humans.
- Existing models show evidence of biphasic disease patterns seen in humans with early replication followed by clearance and immunopathogenic severe disease; however, the disease course is compressed in small animal models.
- Early, but not late, administration of direct acting antivirals/Abs is key for product performance in humans and many small animal models of human disease.
- Success in reversing severe disease signatures later in infection has proven difficult in small animal models and in humans.
- New strategies to extend the therapeutic window for direct acting antivirals and immunotherapeutics are desperately needed.
- What are the most important correlates of protection from SARS-CoV-2 infection and severe disease?

Concerns: Existing Animal Models

- There is widespread acknowledgment that additional small animal models that permit a greater spectra of human-like infection outcomes by SARS-CoV-2 will be highly valuable.
- Few models describe evidence for coagulopathy, hypoxemic respiratory failure, multiple organ failure or death. Continued viral replication and antigen expression should be considered as a potential pathologic mechanism.
- Need for models that capture and allow for testing of therapeutics designed to reverse “long-hauler” phenotypes involving the lung, central nervous system, kidney, heart, etc.
- Availability and facilities to work with some of these models is limited, suggesting that the best choice for routine and widespread use may be the laboratory mouse. More non-human primate models of severe COVID-19 disease are needed.
- Transgenics: Available on one genetic background that does not express human FcγRT. Humanized FcγRT allows for better modeling of human antibody clearance in mice and thus aids antibody and drug development.
- Models do not capture the role of natural genetic variation on SARS-CoV-2 pathogenesis.

- Testing in the majority of animal models has shown that dexamethasone does not reverse disease severity, as has been reported in humans when administered late in disease progression.
- Models are needed: to capture known susceptibility comorbidities like age, diabetes, obesity, and hypertension; and potentially capture rare secondary, post-COVID-19 fungal or bacterial infections.
- Models are needed that are amenable for use with new VoCs as they arise during the pandemic.
- A few mouse models exist for development/testing of broad-based *Sarbecovirus*, *Merbecovirus* and *Coronavirus* antivirals, immunotherapeutics and universal vaccines; however, more are needed including non-human primate and transmission models.

Small Animal Models for New Variants of Concern

- Severe disease animal models are needed for key VoCs, like B.1.351, P.1, B.1.1.7, and B.1.617.
- Many VoCs contain a N501Y mutation that promotes replication in the mouse and disease models are currently under development.
- Elucidate relationships between VoC antigenic variation, reduced neutralization capacity, and therapeutic antibody/vaccine performance *in vivo*.

In vitro *Neutralization Assays*

- Determine relationships between *in vitro* assays and impact of resistance mutations on *in vivo* phenotypes.
- Determine the assay variables that alter neutralization titers.
- Identify the assay variables most relevant for correlating *in vitro* neutralization titers to *in vivo* protection and breakthrough.

Immunotherapeutics and SARS-CoV-2 Neutralizing Antibodies

- Delineate the relationship between therapeutic antibody treatment and post-acute SARS-CoV-2 disease outcomes.
- What new approaches are available to extend the therapeutic window of direct acting antivirals and antibodies?
- What are the most important aspects/features of antibodies for providing long-term protective immunity against SARS-CoV-2?

Biodistribution and Next Generation Delivery Modalities

- What factors regulate the biodistribution of antibodies to the upper and lower respiratory tracts?
- Can the dose of Abs authorized under EUA tolerate a 20X shift in neutralization titers?
- What lessons were learned from treating COVID-19 patients with convalescent plasma?

Discussion Panel

Moderator: Connie Schmaljohn, Ph.D., NIAID, NIH

Panelists: Christopher Ellis, Ph.D., FDA; Tom Hope, Ph.D., Northwestern University; Kevin Saunders, Ph.D., Duke University; Skip Virgin, M.D., Ph.D., Vir Biotechnology; and Emmie de Wit, Ph.D., NIAID, NIH

Dr. Connie Schmaljohn led the Panel discussion on the preclinical delivery, pharmacology and efficacy of anti-SARS-CoV-2 antibodies. Dr. Emmie de Wit suggested that antibodies, especially NAbs, are the most important correlates of protection, especially against SARS-CoV-2 variants. She commented that many animal studies have shown prophylactic effects of anti-SARS-CoV-2

antibodies, and therapeutic administration early after infection has been successful. Although variants need to be taken into consideration, reassuring data show that several antibodies already in use still can prevent severe disease. She added that the T cell component of the immune response is being recognized as important in the vaccinated population because of epitope conservation. Dr. Skip Virgin emphasized that conserved epitopes are the most important aspect of antibodies for providing long-term protective immunity. He commented that the RBD is highly variable under immune selective pressure, so identifying antibodies that bind in other locations likely are the best future path to pursue. Effector functions of antibodies are extremely important in animal models of infection, so Fc-enhanced engineered mAbs are being explored currently in humans. He noted that dose also is an important consideration for the potency of the protective effect. Dr. Kevin Saunders commented on experiments to introduce mutations that increase the Fc affinity for gamma receptors. When asked whether antibodies engineered to interact with Fc gamma receptors have a higher response of anti-drug antibody, Dr. Virgin commented that data on the LS mutation are available, but he did not consider this a major clinical issue. Dr. Saunders added that his research showed that the LS mutation induced comparable anti-drug antibody response to wild type Fc, but the mechanism remains unknown.

Dr. Schmaljohn asked the Panel members about the most important function of antibodies for predicting prophylactic or therapeutic potency. Dr. Saunders commented on the importance of neutralizing potency, explaining that highly potent antibodies lead to low levels of virus replication. He commented that effector functions become more critical as potency increases; however, variants may complicate this understanding.

Dr. Christopher Ellis commented on the use of animal model data to support mAb development, noting that animal models were not available early in the pandemic, so data from studies using animal models often were collected in parallel with clinical trials. He noted that the overall utility of mAb-specific data has been limited to supporting the initial assessment of *in vivo* activity and evaluating the potential for antibody-dependent enhancement of infection. Dr. Virgin emphasized the importance of considering differences between animal models and human biology. Dr. Hope added that a variety of animal models will be needed to address the many health complications that individuals with SARS-CoV-2 will experience in the future.

Dr. Tom Hope outlined studies on the biodistribution of therapeutic antibodies, explaining that antibodies can be identified in tissues within 24 hours, and each has unique characteristics and accumulates in different places in the body. He suggested that the accumulation of each antibody should be studied in addition to its binding mechanisms and other characteristics.

Dr. Schmaljohn inquired whether a model for antibody-dependent disease enhancement is still needed and how to model human sequelae, such as “long COVID”. Dr. de Wit commented that her team reacted as quickly as possible at the beginning of the pandemic to develop and characterize animal models to test vaccine and antiviral candidates. She noted that variants began to emerge before the researchers could focus on SARS-CoV-2 pathogenesis or long COVID, and they began studies on the variants. She emphasized that this will be a long-term project. Dr. Virgin emphasized that it is important to distinguish antibody-mediated enhancement defined by vitro models where you can measure an increased uptake of virus from the more worrisome antibody-mediated enhancement of disease that may happen *in vivo*. He added that significant data were gathered during the pandemic supporting predictions that enhancement of disease mediated by antibodies is not a major problem.

Dr. Schmaljohn and Panel members discussed strategies to address the potential for reduced antibody potency to SARS-CoV-2 variants. Dr. Virgin suggested that the best protection against variants is to focus on conserved epitopes; half-life extension and Fc-effector function, as well

as antibody combinations to help mitigate variants. He added that the concept of the conserved epitope is important for pandemic preparedness—antibodies that are SARS-CoV-2–specific have been helpful, but we should identify and develop antibodies that can protect against other SARS coronaviruses in the future.

Dr. Schmaljohn asked the Panel about the predictive value of current *in vitro* assays. Dr. Saunders commented that certain RBD antibodies mediate virus uptake *in vitro*, but these antibody-dependent enhancements are not found *in vivo*. He noted that antibodies in polyclonal mixtures also lead to different outcomes *in vivo* versus *in vitro*. While pseudovirus assays and laboratory assays correlated well with neutralization titers, Dr. Saunders commented that good concordance has been seen in antibody effector functions in neutralization assays. He suggested that a mixture of antibody functions will contribute most to protection.

Dr. Ellis explained that the FDA is concerned about the potential clinical impact of mAbs with reduced susceptibility to variants from a regulatory perspective. He commented that insufficient data are currently available on circulating variants to determine the clinical impact on the use of mAbs to potentially prevent SARS-CoV-2 infection and to treat mild to moderate COVID-19.

Dr. Schmaljohn asked the Panel about the potential of targeting antibodies to specific tissues or fluids. Dr. Hope commented that while studies suggest targeting will be possible, additional research is needed to identify the specific aspects of each antibody that affect its ability to target mucosal fluids. He added that his team has developed a probe to identify sites of coronavirus replication in the macaque, which will provide useful information to better understand long COVID-19. Dr. Hope commented that nucleic acid delivery of antibody requires strong confidence in the antibody used as it will be present for a significant amount of time and could be problematic if the virus mutates.

Session III: Real-World Clinical Use of Anti-SARS-CoV-2 Antibodies

Presenter: Katharine Bar, M.D., University of Pennsylvania

Dr. Katharine Bar presented on the clinical use of anti-SARS-CoV-2 antibodies in real world settings. Since the identification of the first SARS-CoV-2 infections in late 2019, the COVID-19 pandemic has caused more than 175 million cases worldwide,[13] while ravaging global economies, and overwhelming countless social support systems. Antibodies against SARS-CoV-2, in the form of convalescent plasma and related products as well as monoclonal antibodies, were identified early as potential prevention and therapy strategies. The clinical development, testing for efficacy, and implementation of these antibody-based strategies have been high priorities, with demonstrated accomplishments and challenges.

Tension Between the Pace of the Pandemic and Generation of High-Quality Evidence

The COVID-19 pandemic emerged quickly in late 2019 and has continued to evolve rapidly over 2020 and 2021. The scientific community's understanding of how the virus is spread, the mechanisms of pathophysiology, risk factors for severe illness, and best practices for prevention and treatment have changed at an unprecedented pace. Successive waves of cases with shifting demographics have challenged health care capacity, while institutions have diligently sought to employ appropriate infection control and patient care practices. In the U.S., the development and distribution of safe and effective vaccines have led to decreases in cases and deaths, while the specter of increasing diversity and prevalence of VoCs grows.

The biomedical community responded on many fronts to develop strategies to diagnose, prevent, and treat COVID-19. Antibodies against SARS-CoV-2, in the form of convalescent plasma and related products, as well as monoclonal antibodies, were identified early as potential prevention and therapy strategies. Two types of antibody preparations, COVID-19 convalescent plasma (CCP) and commercially developed monoclonal antibodies, highlight distinct development and efficacy testing pathways.

COVID-19 Convalescent Plasma: Extensive Use, Unclear Evidence of Benefit

Early in the COVID-19 pandemic, CCP was recognized as a potentially promising intervention. Use of convalescent plasma in other infectious diseases[85, 86] and previous coronavirus pandemics[87, 88] provided biological plausibility, and early observational studies suggested possible benefit.[89-91] In the setting of limited alternative treatments and desperate clinical need, CCP was widely used in hospitalized COVID-19 patients in the U.S. via an expanded access program (EAP) or emergency use authorization (EUA).[92, 93] These programs enabled access to CCP to more than 500,000 hospitalized individuals, with up to 40% of US COVID-19 inpatients receiving CCP in the fall of 2020.[94] Observational analyses of a sub-cohort of hospitalized CCP recipients from the U.S. FDA's Expanded Access Program suggested possible benefit in recipients of early, high-titer plasma.[95] These programs also made the implementation of well-powered, randomized clinical trials highly challenging in the U.S. Only recently have reports from larger, randomized controlled trials conducted largely outside the U.S. provided more robust assessments of the efficacy of CCP.[96-100] In total, results suggest CCP is not highly efficacious when given to general cohorts of hospitalized COVID-19 patients. Many of the large trials; however, have substantial heterogeneity in three key factors: *i*) the antibody titer and number of units of donor plasma; *ii*) the timing of plasma administration; and *iii*) the baseline comorbidities of patients that influence the risk for severe COVID-19 and effective immune responses. In particular, immunocompromised individuals who are unable to mount their own antibody responses may have greater benefit from CCP.[101] Some smaller studies, subset analyses within the larger randomized trials, and metanalyses have suggested possible benefit with earlier treatment, higher titer plasma, in highly co-morbid patient populations.[102, 103] Pending data from ongoing trials, including large outpatient trials and compilation projects attempting to parse through the heterogeneous treatment effects, may further delineate if CCP has benefit at early timepoints and/or in specific individuals, thus suggesting use of CCP via a more personalized medicine approach. Currently, the EUA for high-titer plasma in hospitalized patients who are early in their disease course and/or immunocompromised remains available, but the efficacy and best approach to use of CCP remains unclear.

Anti-SARS-CoV-2 mAbs: Accumulating Efficacy Data

Highly potent, broadly neutralizing monoclonal antibodies were identified in SARS-CoV-2-infected patients early in the pandemic.[104] Collaborative NIH and industry trials have demonstrated efficacy in treatment of early disease by mAbs,[6, 8] resulting in EUA for single and combination mAbs to individuals beginning in November 2020. As of early June 2021, three distinct products (Bamlanivimab/Etesevimab IV, REGEN-COV (Casirivimab/Imdevimab) IV and SC, and Sotrovimab IV) have emergency use authorization for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (≥12 years of age and ≥ 40kg) with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19. Notably, mAb therapies are only authorized for outpatients who can be treated within 10 days of symptom onset. The current mAb products are not authorized for hospitalized patients; recently reported data from the RECOVERY trial[105] indicating a possible mortality benefit for mAb use in immunocompromised patients hospitalized with COVID-19 will be evaluated in the near future.

These mAbs are now widely available free of charge in the U.S. on request and may be ordered by any qualified site by emailing covid19therapeutics@hhs.gov. Just over one million doses have been shipped to approximately 3,500 unique sites in the U.S. Over 500,000 patient courses have been administered. Sites have included hospital systems, dialysis centers, American Indian/Alaska Native Health, home infusions, mobile units, long-term care facilities, nursing homes, the Corrections system, Federally Qualified Health Centers, and others. Sites are responsible for identifying appropriate patients and administering the mAbs.

An interagency group from ASPR, NIH, FDA, and CDC monitors the distribution of VoCs by state on a weekly basis and makes decisions regarding future distribution of ordered mAbs. Recently, virus surveillance revealed increased prevalence of VoCs (P.1 and B.1.351), which prompted a pause in distribution of bamlanivimab/etesevimab to nine states and raised concerns in other regions.

In addition to treatment, mAbs have shown promise for prevention, but are not currently authorized for emergency use for this indication. Data for the prevention of SARS-CoV-2 infection and COVID-19 in high-risk individuals like nursing home residents and staff and household contacts of infected individuals[4, 28] have been presented publicly, and potential EUA for prevention is under review by the FDA. Strategies for implementation of mAbs for prevention, however, have not yet been determined.

Challenges to Conduct and Completion of Clinical Trials of Anti-SARS-CoV-2 mAbs

The pandemic prompted unprecedented partnerships and effort to test mAbs for treatment of outpatients, and inpatients, as well as for prevention. Coordination through industry partnerships with the NIH, the COVPN, and other governmental agencies enabled the platform trials of ACTIV-2, ACTIV-3, and prevention studies of the COVPN. These successful programs were the result of huge expenditures of resources and collaboration between many groups and individuals. Many challenges to trial implementation were overcome in order to enroll and conduct these studies. Specific challenges identified by investigators include: mixed messages to potential participants to stay home vs. seek treatment; challenges of identifying patients within the narrow treatment window just after testing positive; locating appropriate facilities to administer antibodies in the outpatient setting with adequate space to enable distancing and patient flow and adequate staffing; prioritizing outpatient trials in the setting of competing studies, including inpatient treatment trials and vaccine studies, and EUA of experimental interventions; creating centralized and timely messaging; staff work burden and burnout; recruitment in underrepresented or disenfranchised communities. Solutions to these challenges required focused attention and creative thinking. Investigators in these networks highlight the need to maintain support of these collaborative trial infrastructures, as additional trials will be needed for potential future COVID-19 surges due to SARS-CoV-2 variants and future pandemics due to other pathogens.

Challenges to Implementation of Anti-SARS-CoV-2 mAbs

Despite demonstration of substantial efficacy in treatment and EUA indications for mAbs since November 2020, widespread use of mAbs has been limited due to the existence of multiple barriers to broad administration.

Timely Identification of Eligible COVID-19 Patients and Linkage to a Treatment Facility

The current EUA for three mAb options allows use of three mAb products to treat individuals diagnosed with SARS-CoV-2 infection with mild to moderate COVID-19 who are at high risk for

development of severe disease within 10 days of symptom onset. Thus, there is a narrow window of opportunity in which to identify eligible patients in the early infection period and link them to mAb treatment. One substantial obstacle to rapid treatment is the disconnect between locations of testing, for example acute care centers, employee testing programs, and emergency departments, and providers who are aware of mAb indications and able to prescribe or refer for mAb therapy. Once aware of a new positive test, providers must understand the indications for mAbs, and believe that use will be beneficial for their patients. Efforts to better educate providers across primary care providers, acute care providers, and infectious disease specialists are needed to better inform them of the potential benefits and available safety data of mAbs in COVID-19 patients indicated with current EUA. Next, providers must contact eligible patients in the early phase of disease prior to development of a severe disease, be able to adequately describe the risks and benefits of EUA mAbs and provide an accessible pathway to treatment. Providers must be made aware of facilities that can administer the mAbs. Given the relatively few agencies providing these services, community outreach is needed to connect with providers of potential patients not directly affiliated with specific hospital systems or treating agency. Referral systems that reach community health centers, private physician networks, and health departments are needed.

Patient Perceptions of Anti-SARS-CoV-2 mAbs

Patients may have misgivings about receiving treatments during early COVID-19 disease, when mAbs are most effective.[8, 28] Providers must be able to discuss perceptions about the potential risks of COVID-19, the risk to benefit balance of EUA therapies, and risks of to personal health or of virus transmission with traveling to a health care facility. Recommendations to leave their home to receive treatment may contradict messaging they have previously received. Thus, generalized messaging to the public on the availability and potential benefits of mAb therapy could be beneficial, but direct discussions with known and trusted providers may be required to facilitate use of mAbs during the early treatment period.

Treatment Facility Staffing and Infrastructure Requirements

The administering facility must provide dedicated staff, including providers to complete EUA consent and observation, pharmacists to prepare the antibodies, and nurses to administer and monitor the patient through the process. Facilities available for COVID-19 positive patients to receive intravenous infusions are required, which must be factored into healthcare systems' approaches, including appropriate personal protective equipment, ventilation, transportation, and follow up of patients who receive infusions if not currently followed by a health system provider. Infusions take several hours, with time for pharmacy preparation, infusion and post-infusion observation; thus, the number of patients who can be treated per day is limited. The recent authorization of subcutaneous administration as an option,[106] may alleviate some of the logistical burdens. These staffing and infrastructure requirements entail substantial financial commitments from participating hospital systems.

Equitable Use of Anti-SARS-CoV-2 mAbs, Within the U.S. and Internationally

Substantial efforts are needed to provide information about anti-SARS-CoV-2 mAbs to all eligible patients within the United States. Multiple structural healthcare and systemic inequities have caused disproportionate case rates, as well as morbidity and mortality in minority populations, including African Americans, Latinx, and Native American communities. Many vulnerable individuals do not have primary care physicians who can advocate for mAb use, help navigate the referral process, and explain the financial supports available. Additional efforts at outreach and education on the

availability, potential benefits, and means to access mAbs are required to provide this treatment option equitably. Outreach across providers in these areas, patients, and the general population may be beneficial. In addition, language barriers may make conveyance nuanced information challenging, thus fluent providers or interpreter services are important.

Real and/or perceived financial barriers to transportation and/or facility fees to access government supplied mAbs may be issues currently. For impending commercially-available mAbs, use and communication of strategies to provide mAbs to the uninsured and underinsured, like the U.S. Health Resources and Services Administration (HRSA)-run COVID-19 Uninsured Patient Program,[107] are vital.

While case rates are currently falling in the U.S., the pandemic continues unabated across the globe. In addition to rapid production and distribution of vaccines to all countries with ongoing epidemics, efforts to supply anti-SARS-CoV-2 mAbs to the global community should be considered.

Challenges From Variants of Concern (VoCs)

National and regional surveillance are essential for current use of mAbs, as well as understanding of vaccine protection. National surveillance recently identified increased prevalence of several variants with resistance to some mAbs, prompting pause in distribution of Bamlanivimab/Etesevimab to eight states (FDA); other regions have altered prescribing patterns based on local surveillance. The remaining two mAb formulations appear potent against VoCs, but continued vigilance is needed. At the University of Pennsylvania, local surveillance revealed circulation of VoCs in early 2021.[108] With these sequencing data, local hospital pharmacy committees were able to shift to only administering the mAbs that retained activity against all circulating variants. Regions and communities without active surveillance programs may be unaware of the frequency and specific types of VoCs circulating in their communities. Efforts to increase the breadth of surveillance and communicate these results to distributing agencies, providers, and the public will be important. In the meantime, regions without rapid, local surveillance may need to err on the side of caution and prescribe mAbs that retain potency against all or most VoCs.

Vulnerable Populations

Vulnerable populations, including immunocompromised patients with severe COVID-19 may have options of high-titer CCP through EUA or mAbs through compassionate use or similar programs,[101] though the logistics of obtaining these products are often prohibitively challenging.

Given the supportive data from clinical trials of prevention, potential approval for EUA use is anticipated. Anti-SARS-CoV-2 would likely be indicated in not-yet-vaccinated individuals or in people who are not able to fully respond to vaccination. While the extent of protection gained from vaccination is unclear, the increased morbidity and mortality of COVID-19 in cancer patients and others with immunodeficiency merits serious consideration of prophylactic administration of mAbs or other antibody products.[109] Clinical trials, as well as patient registries, pragmatic trials and real-world evidence in these vulnerable populations may be informative.

Importance of Momentum: Maintaining Infrastructure, Virus Surveillance, and Messaging

Combatting COVID-19 has required huge expenditures of resources and capital, as well as building unprecedented collaborations. As the U.S. epidemic wanes in the face of increased vaccine uptake, it is important to retain the relationships and infrastructure built over the past 16 months. As we encounter increasing frequency and diversity of VoCs, as well as new pandemics in years to come, it is vital to continue virus surveillance, identification and development of new mAbs, and innovate

with novel diagnostics (e.g., point of care resistance testing) and methods to administer therapies (e.g., lower volumes, subcutaneous, self-administered options). It is similarly important to continue to support and develop the clinical trials framework with pragmatic trial structures to efficiently test new mAbs for prevention and treatment indications and continue evaluations through real world evidence gathering processes (https://www.fda.gov/media/108510/download).

Misperceptions about SARS-CoV-2, COVID-19, and available approaches to prevention and treatment remain, and are key obstacles to effective prevention and treatment. For mAb use, specific messaging and education on the availability and potential benefits of mAbs for prevention and treatment could facilitate more widespread use. Dissemination of updated information on internationally, nationally and regionally circulating variants, and their impact on available antibody treatments will be critical. Education and messaging directed towards providers, specific populations who are disenfranchised from health services or heavily affected by COVID-19, and the general population are needed.

Discussion Panel

Moderator: Rajesh Gandhi, M.D., Massachusetts General Hospital

Panelists: Ada Adimora, M.D., M.P.H., University of North Carolina at Chapel Hill; Judith Currier, M.D., University of California, Los Angeles; Fred Korley, M.D., Ph.D., University of Michigan; Meagan O'Brien, M.D., Regeneron; John Redd, M.D., M.P.H., FACP, Office of the Assistant Secretary for Preparedness and Response, U.S. Department of Health and Human Services; and Mark Williams, M.D., FCCM, FCCP, Lilly

Dr. Rajesh Gandhi led the Panel discussion addressing real-world clinical use of anti-SARS-CoV-2 antibodies. Dr. Judith Currier commented on the challenges of setting up the infrastructure for clinical trials in the outpatient setting, which needs to occur where the participant can be seen safely from an infection control perspective and that also has appropriate clinical staff. Many outpatient sites added trailers or tents, but staffing was not increased in parallel. Dr. Currier described the difficulties of finding sufficient resources for the outpatient setting when severe hospital cases were prioritized. She also observed that many SARS-CoV-2 testing sites were physically separate from treatment and clinical trials sites. Dr. Currier commented that co-localizing or bringing testing and clinical sites into proximity might speed the roll-out of treatment and research studies.

Dr. Gandhi and the Panel members commented on the importance of understanding local challenges to successful implement of clinical trials. He reported that outpatient clinical trials in large academic centers in Boston were not near the centers of the epidemic. Dr. Currier commented on the difficulties of transportation for trial participants and added that community health centers typically do not have sufficient clinical staff to conduct trials and resources are key to successful implementation of clinical studies. Dr. Mark Williams described the success of mobile emergency medical services units that could travel to patients and help with transport to clinical centers and home infusions. He noted the success of United Health Group in providing home infusions early in the pandemic.

Dr. John Redd noted the differences between the "clinical trial" phase of the pandemic and the "post-EUA" phase—noting that the level of evidence required by the FDA for EUAs is different than that needed for full approval of a treatment. He commented that equitable methods for deciding who is prioritized for treatment and distributing therapeutic agents are needed. He added that these issues are common to EUAs but were exacerbated by requiring new infrastructure.

Dr. Fred Korley commented on the challenge of messaging when public health guidance changes

rapidly during the ongoing pandemic, explaining that one challenge for clinicians is to communicate clearly to the public that the pandemic is an unprecedented event in which guidance will change over time as additional scientific and clinical data become available. He emphasized the importance of messaging. Dr. Gandhi added that many patients look to trusted clinicians for guidance rather than to centralized referral systems, which is why providing clinicians' education on the latest advances is critical to providing the best care possible.

Dr. Redd noted the importance of rapid SARS-CoV-2 antigen tests, which can allow people who test positive to receive treatment quickly and in the same location where the tests are administered. Dr. Ada Adimora pointed out that many primary care clinicians need to be aware that their patient has received a test by another provider or clinics, and they need to be aware how to coordinate where patients can receive antibody treatments. Clinician education programs can assist in ensuring that health care providers have the latest information with which to provide optimum care for their patients. Dr. Adimora also highlighted additional barriers to equitable implementation including language, especially given the nuance required to communicate about the pandemic, and the numerous challenges related to socioeconomic status or the combination of race and ethnicity with socioeconomic status. She noted that geographic and transportation barriers to health care systems can be significant, with many infusion centers for administering antibodies located far from the patient's home or the site of diagnosis. Patients also may have inadequate insurance or no primary care provider to provide referrals for treatment. Dr. Adimora commented that more equitable distribution of health care could be improved with the use of intramuscular injection or subcutaneous administration of mAbs or point-of-care infusions, as well as wide dissemination of clinical education and information on referral networks. Dr. Korley commented that early clinical studies were small in sample size and results often had a high fragility index. Providers who consult the literature may be unimpressed by the initial level of evidence, and many likely did not consult later data which greatly strengthened the justification for the treatments.

Dr. Meagan O'Brien pointed out that the decision to use subcutaneous administration was supported by data from a dose-finding study that found similar viral clearance at all dosages. The scientific data for subcutaneous administration of antibodies are encouraging and has shown that subcutaneous administration prevents progression to symptomatic infection and reduces duration of symptoms in some studies. Dr. Redd commented that co-formulated REGEN-COV has been successful in both subcutaneous and intravenous administration.

Dr. Gandhi asked the Panel members whether antibody treatments could be decreased to a single self-administered injection. Dr. O'Brien responded that many opportunities exist to improve current administration methods, but researchers have been prioritizing efficacy. The emergence of SARS-CoV-2 variants suggests that coformulations should be considered. She emphasized that these are still the first generation of SARS-CoV-2 treatments, so many advances are likely in the future.

When Dr. Gandhi asked about the federal government's role in distribution, Dr. Redd commented that the government has an ethical obligation to ensure that safe and effective treatments are available equally to everyone in the United States and subsequently the world. Some prioritization of access to treatment were implemented when limited supply of antibodies conditions were in place; however, this has subsequently improved. He also commented on the importance of geographic and temporal equity, so treatment availability must be stable.

Dr. Gandhi inquired about how to conduct real-time variant surveillance. Dr. Korley commented that a personalized approach to treatment could be developed soon, which would ensure that the right

patient receives the right antibody therapy at the right time. A gold standard antibody neutralization test could allow the consideration of other markers strongly correlated with antibody levels, such as inflammatory tests. Rapid point-of-care SARS-CoV-2 diagnostic tests are needed to better identify the variants and ensure that the correct mAb is provided. Dr. Gandhi commented that although the field has advanced significantly over the course of the pandemic, much remains to be done to achieve personalized treatment.

Dr. Gandhi and the Panel discussed prevention and treatment options using antibodies for immunocompromised patients and those who have not responded to the vaccine. They noted that REGEN-COV and sotrovimab are effective against current VoCs. Dr. O'Brien encouraged the use of combination therapy. Patients without strong immune responses to COVID-19 vaccination may benefit from antibodies as a prevention strategy, but more data are needed.

Dr. Gandhi asked about the future of antibody treatments and the Panel members emphasized the importance of developing second-generation antibodies, improving ease of administration, and making therapies widely available around the world.

Wrap Up and Closing Comments

Adrian McDermott, NIAID, NIH; Connie Schmaljohn, NIAID, NIH; and Rajesh Gandhi, Massachusetts General Hospital

Panel moderators summarized the discussions in their individual sessions. They emphasized that much has been learned during the pandemic; much remains to be learned. They identified several areas of further research on antibodies and their potential use in treatment and prevention, including targeting conserved regions, improving animal models, and engineering better antibodies. Dr. Boggiano closed the Summit by thanking the presenters, session moderators, and participants. He noted that the entire summit will be archived on the NIH website and a meeting summary also will be available on the website. Dr. Boggiano then adjourned the meeting.

References

1 Salazar E, Christensen PA, Graviss EA, Nguyen DT, Castillo B, Chen J, et al. Treatment of coronavirus disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality. *Am J Pathol*. 2020;190(11):2290-303.

2 Foundation for the National Institutes of Health. https://fnih.org/our-programs/ACTIV. Accessed on August 29, 2021.

3 Cohen MS, Nirula A, Mulligan MJ, Novak RM, Marovich M, Yen C, et al. Effect of bamlanivimab vs placebo on incidence of COVID-19 among residents and staff of skilled nursing and assisted living facilities: a randomized clinical trial. *JAMA*. 2021;326(1):46-55.

4 OBrien MP, Neto EF, Chen K-C, Isa F, Heirman I, Sarkar N, et al. Casirivimab with imdevimab antibody cocktail for COVID-19: interim results. In: CROI Foundation/IAS-USA, ed. Conference on Retroviruses and Opportunistic Infections 2021. Virtual; 2021:35.

5 Chen P, Nirula A, Heller B, Gottlieb RL, Boscia J, Morris J, et al. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with COVID-19. *N Engl J Med*. 2021;384(3):229-37.

6 Gottlieb RL, Nirula A, Chen P, Boscia J, Heller B, Morris J, et al. Effect of bamlanivimab as monotherapy or in combination with etesevimab on viral load in patients with mild to moderate COVID-19: a randomized clinical trial. *JAMA*. 2021;325(7):632-44.

7 Dougan M, Nirula A, Gottlieb RL, Azizad M, Mocherla B, Chen P, et al. Bamlanivimab+ etesevimab for treatment of COVID-19 in high-risk ambulatory patients. Conference on Retroviruses and Opportunistic Infections 2021. Virtual; 2021.

8 Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with COVID-19. *N Engl J Med*. 2021;384(3):238-51.

9 Regeneron Pharmaceuticals I. Phase 3 trial shows REGEN-COV™ (casirivimab with imdevimab) antibody cocktail reduced hospitalization or death by 70% in non-hospitalized patients. 2021.

10 U.S. Food and Drug Administration. FDA EUA for REGEN-COV. 2021.

11 ACTIV-3/TICO LY-CoV555 Study Group. A neutralizing monoclonal antibody for hospitalized patients with COVID-19. *N Engl J Med*. 2020;384(10):905-14.

12 GISAID. https://www.gisaid.org. Accessed on July 9, 2021.

13 World Health Organization. Coronavirus disease (COVID-19) weekly epidemiological update and weekly operational update. 2021.

14 Wang X. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N Engl J Med*. 2021;384(16):1577-8.

15 Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-16.

16 Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against COVID-19. *N Engl J Med*. 2021;384(23):2187-201.

17 Knoll MD, Wonodi C. Oxford-AstraZeneca COVID-19 vaccine efficacy. *Lancet*. 2021;397(10269):72-4.

18 Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science*. 2020;370(6522):1339-43.

19 Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*. 2020;367(6485):1444-8.

20 Hoffmann M, Kleine-Weber H, Pöhlmann S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell*. 2020;78(4):779-84.e5.

21 Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature*. 2020;581(7807):215-20.

22 Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181(2):271-80.e8.

23 Amanat F, Krammer F. SARS-CoV-2 vaccines: status report. *Immunity*. 2020;52(4):583-9.

[24] Hurt AC, Wheatley AK. Neutralizing antibody therapeutics for COVID-19. *Viruses*. 2021;13(4).

[25] The Antibody Society. https://www.antibodysociety.org/covid-19-biologics-tracker. Accessed on July 11, 2021.

[26] Taylor PC, Adams AC, Hufford MM, de la Torre I, Winthrop K, Gottlieb RL. Neutralizing monoclonal antibodies for treatment of COVID-19. *Nat Rev Immunol*. 2021;21(6):382-93.

[27] Hansen J, Baum A, Pascal KE, Russo V, Giordano S, Wloga E, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science*. 2020;369(6506):1010-4.

[28] Cohen MS, Nirula A, Mulligan MJ, Novak RM, Marovich M, Yen C, et al. Effect of bamlanivimab vs placebo on incidence of COVID-19 Among residents and staff of skilled nursing and assisted living facilities: a randomized clinical trial. *JAMA*. 2021;326(1):46-55.

[29] Wu X, Li N, Wang G, Liu W, Yu J, Cao G, et al. Tolerability, safety, pharmacokinetics, and immunogenicity of a novel SARS-CoV-2 neutralizing antibody, etesevimab in chinese healthy adults: a randomized, double-blind, placebo-controlled, first-in-human phase 1 study. *Antimicrob Agents Chemother*. 2021.

[30] Pinto D, Park YJ, Beltramello M, Walls AC, Tortorici MA, Bianchi S, et al. Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature*. 2020;583(7815):290-5.

[31] Collins FC, Woodcock J, Graham BS, Arvin A, Bieniasz P, Ho D, et al. Therapeutic neutralizing monoclonal antibodies: report of a summit sponsored by Operation Warp Speed and the National Institutes of Health. Therapeutic Neutralizing Monoclonal Antibodies for COVID-19 Scientific Summit, August 20, 2020 2020. Virtual. 2020.

[32] LaJolla Institute for Immunology. CoVIC. 2021.

[33] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579(7798):265-9.

[34] Austin CC, Bernier A, Bezuidenhout L, Bicarregui J, Biro T, Cambon-Thomsen A, et al. Fostering global data sharing: highlighting the recommendations of the Research Data Alliance COVID-19 working group. *Wellcome Open Res*. 2020;5:267.

[35] Hoffmann M, Zhang L, Krüger N, Graichen L, Kleine-Weber H, Hofmann-Winkler H, et al. SARS-CoV-2 mutations acquired in mink reduce antibody-mediated neutralization. *Cell Rep*. 2021;35(3):109017.

[36] Oude Munnink BB, Sikkema RS, Nieuwenhuijse DF, Molenaar RJ, Munger E, Molenkamp R, et al. Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*. 2021;371(6525):172-7.

[37] Avanzato VA, Matson MJ, Seifert SN, Pryce R, Williamson BN, Anzick SL, et al. Case Study: Prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. *Cell*. 2020;183(7):1901-12.e9.

[38] Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N Engl J Med*. 2020;383(23):2291-3.

[39] Kemp SA, Collier DA, Datir RP, Ferreira I, Gayed S, Jahun A, et al. SARS-CoV-2 evolution during treatment of chronic infection. *Nature*. 2021;592(7853):277-82.

[40] Naveca F, costa c, Nascimento V, souza v, Corado A, Costa Filho R. SARS-CoV-2 reinfection by the new variant of concern (VoC) P.1 in Amazonas, Brazil. Available at https://virological.org/t/sars-cov-2-reinfection-by-the-new-variant-of-concern-voc-p-1-in-amazonas-brazil/596. 2021.

[41] Nonaka CKV, Franco MM, Gräf T, de Lorenzo Barcia CA, de Ávila Mendonça RN, de Sousa KAF, et al. Genomic evidence of SARS-CoV-2 reinfection involving E484K spike mutation, Brazil. *Emerg Infect Dis*. 2021;27(5):1522-4.

[42] O'Toole Á, Hill V, Pybus OG, Watts A, Bogoch, II, Khan K, et al. Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2. *Wellcome Open Res*. 2021;6:121.

[43] Leung K, Shum MH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Euro Surveill*. 2021;26(1).

[44] Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*. 2021;592(7854):438-43.

[45] Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al. Genomics and epidemiology of

the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*. 2021;372(6544):815-21.

46 Liu J, Liu Y, Xia H, Zou J, Weaver SC, Swanson KA, et al. BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants. *Nature*. 2021.

47 Yuan M, Huang D, Lee CD, Wu NC, Jackson AM, Zhu X, et al. Structural and functional ramifications of antigenic drift in recent SARS-CoV-2 variants. *Science*. 2021.

48 Chen J, Wang R, Wang M, Wei GW. Mutations strengthened SARS-CoV-2 infectivity. *J Mol Biol*. 2020;432(19):5212-26.

49 Hodcroft EB, Zuber M, Nadeau S, Vaughan TG, Crawford KHD, Althaus CL, et al. Spread of a SARS-CoV-2 variant through Europe in the summer of 2020. *Nature*. 2021.

50 Thomson EC, Rosen LE, Shepherd JG, Spreafico R, da Silva Filipe A, Wojcechowskyj JA, et al. Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. *Cell*. 2021;184(5):1171-87.e20.

51 Hsieh CL, Goldsmith JA, Schaub JM, DiVenere AM, Kuo HC, Javanmardi K, et al. Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. *Science*. 2020;369(6510):1501-5.

52 Barnes CO, Jette CA, Abernathy ME, Dam KA, Esswein SR, Gristick HB, et al. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. *Nature*. 2020;588(7839):682-7.

53 Robbiani DF, Gaebler C, Muecksch F, Lorenzi JCC, Wang Z, Cho A, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature*. 2020;584(7821):437-42.

54 Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, et al. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect. 2020;9(1):382-5.

55 Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature*. 2021;593(7857):130-5.

56 Liu H, Wu NC, Yuan M, Bangaru S, Torres JL, Caniels TG, et al. Cross-neutralization of a SARS-CoV-2 antibody to a functionally conserved site is mediated by avidity. *Immunity*. 2020;53(6):1272-80.e5.

57 Yuan M, Liu H, Wu NC, Lee CD, Zhu X, Zhao F, et al. Structural basis of a shared antibody response to SARS-CoV-2. *Science*. 2020;369(6507):1119-23.

58 Zhou D, Duyvesteyn HME, Chen CP, Huang CG, Chen TH, Shih SR, et al. Structural basis for the neutralization of SARS-CoV-2 by an antibody from a convalescent patient. *Nat Struct Mol Biol*. 2020;27(10):950-8.

59 Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature*. 2020;584(7821):450-6.

60 McCallum M, De Marco A, Lempp FA, Tortorici MA, Pinto D, Walls AC, et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell*. 2021;184(9):2332-47.e16.

61 Huo J, Zhao Y, Ren J, Zhou D, Duyvesteyn HME, Ginn HM, et al. Neutralization of SARS-CoV-2 by destruction of the prefusion spike. *Cell Host Microbe*. 2020;28(3):497.

62 Whitt MA. Generation of VSV pseudotypes using recombinant ΔG-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J Virol Methods*. 2010;169(2):365-74.

63 Xie X, Muruato A, Lokugamage KG, Narayanan K, Zhang X, Zou J, et al. An infectious cDNA clone of SARS-CoV-2. *Cell Host Microbe*. 2020;27(5):841-8.e3.

64 Muruato AE, Fontes-Garfias CR, Ren P, Garcia-Blanco MA, Menachery VD, Xie X, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. *Nat Commun*. 2020;11(1):4059.

65 Hou YJ, Chiba S, Halfmann P, Ehre C, Kuroda M, Dinnon KH, 3rd, et al. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission *in vivo*. *Science*. 2020;370(6523):1464-8.

66 Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell*. 2020;182(4):812-27.e19.

67 Chung AW, Kumar MP, Arnold KB, Yu WH, Schoen MK, Dunphy LJ, et al. Dissecting polyclonal vaccine-induced humoral immunity against HIV using systems serology. *Cell*. 2015;163(4):988-98.

68 Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, et al. A functional role for antibodies in tuberculosis. *Cell*. 2016;167(2):433-43.e14.

[69] Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. *Nat Rev Immunol*. 2018;18(1):46-61.

[70] Ackerman ME, Moldt B, Wyatt RT, Dugast AS, McAndrew E, Tsoukas S, et al. A robust, high-throughput assay to determine the phagocytic activity of clinical antibody samples. *J Immunol Methods*. 2011;366(1-2):8-19.

[71] Gunn B, Schneider J, Shansab M, Bastian AR, Fahrbach K, Smith At, et al. Enhanced binding of antibodies generated during chronic HIV infection to mucus component MUC16. Mucosal Immunol. 2016;9(6):1549-58.

[72] Jegaskanda S, Job ER, Kramski M, Laurie K, Isitman G, de Rose R, et al. Cross-reactive influenza-specific antibody-dependent cellular cytotoxicity antibodies in the absence of neutralizing antibodies. *J Immunol*. 2013;190(4):1837-48.

[73] Gilchuk P, Murin CD, Milligan JC, Cross RW, Mire CE, Ilinykh PA, et al. Analysis of a therapeutic antibody cocktail reveals determinants for cooperative and broad ebolavirus neutralization. *Immunity*. 2020;52(2):388-403.e12.

[74] Kuzmina NA, Younan P, Gilchuk P, Santos RI, Flyak AI, Ilinykh PA, et al. Antibody-dependent enhancement of ebola virus infection by human antibodies isolated from survivors. *Cell Rep*. 2018;24(7):1802-15.e5.

[75] Gilchuk P, Kuzmina N, Ilinykh PA, Huang K, Gunn BM, Bryan A, et al. Multifunctional pan-ebolavirus antibody recognizes a site of broad vulnerability on the ebolavirus glycoprotein. *Immunity*. 2018;49(2):363-74.e10.

[76] McCray PB, Jr., Pewe L, Wohlford-Lenane C, Hickey M, Manzel L, Shi L, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. *J Virol*. 2007;81(2):813-21.

[77] Avery LB, Wang M, Kavosi MS, Joyce A, Kurz JC, Fan YY, et al. Utility of a human FcRn transgenic mouse model in drug discovery for early assessment and prediction of human pharmacokinetics of monoclonal antibodies. *MAbs*. 2016;8(6):1064-78.

[78] Giragossian C, Clark T, Piché-Nicholas N, Bowman CJ. Neonatal Fc receptor and its role in the absorption, distribution, metabolism and excretion of immunoglobulin G-based biotherapeutics. *Curr Drug Metab*. 2013;14(7):764-90.

[79] Proetzel G, Roopenian DC. Humanized FcRn mouse models for evaluating pharmacokinetics of human IgG antibodies. *Methods*. 2014;65(1):148-53.

[80] Proetzel G, Wiles MV, Roopenian DC. Genetically engineered humanized mouse models for preclinical antibody studies. *BioDrugs*. 2014;28(2):171-80.

[81] Tam SH, McCarthy SG, Brosnan K, Goldberg KM, Scallon BJ. Correlations between pharmacokinetics of IgG antibodies in primates vs. FcRn-transgenic mice reveal a rodent model with predictive capabilities. *MAbs*. 2013;5(3):397-405.

[82] Schlothauer T, Herter S, Koller CF, Grau-Richards S, Steinhart V, Spick C, et al. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. *Protein Eng Des Sel*. 2016;29(10):457-66.

[83] Baker K, Rath T, Pyzik M, Blumberg RS. The role of FcRn in antigen presentation. *Front Immunol*. 2014;5:408.

[84] Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I, Blumberg RS. The neonatal Fc receptor (FcRn): a misnomer? *Front Immunol*. 2019;10:1540.

[85] Mair-Jenkins J, Saavedra-Campos M, Baillie JK, Cleary P, Khaw FM, Lim WS, et al. The effectiveness of convalescent plasma and hyperimmune immunoglobulin for the treatment of severe acute respiratory infections of viral etiology: a systematic review and exploratory meta-analysis. *J Infect Dis*. 2015;211(1):80-90.

[86] Rinaldo CR, Jr. Passive immunization against poliomyelitis: the Hammon gamma globulin field trials, 1951–1953. *Am J Public Health*. 2005;95(5):790-9.

[87] Soo YO, Cheng Y, Wong R, Hui DS, Lee CK, Tsang KK, et al. Retrospective comparison of convalescent plasma with continuing high-dose methylprednisolone treatment in SARS patients. *Clin Microbiol Infect*. 2004;10(7):676-8.

[88] Cheng Y, Wong R, Soo YO, Wong WS, Lee CK, Ng MH, et al. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis*. 2005;24(1):44-6.

[89] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe

COVID-19 patients. *Proc Natl Acad Sci U S A*. 2020;117(17):9490-6.

90 Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA*. 2020;323(16):1582-9.

91 Zeng QL, Yu ZJ, Gou JJ, Li GM, Ma SH, Zhang GF, et al. Effect of convalescent plasma therapy on viral shedding and survival in patients with coronavirus disease 2019. *J Infect Dis*. 2020;222(1):38-43.

92 Joyner MJ, Wright RS, Fairweather D, Senefeld JW, Bruno KA, Klassen SA, et al. Early safety indicators of COVID-19 convalescent plasma in 5,000 patients. *J Clin Invest*. 2020;130(9):4791-7.

93 Senefeld JW, Johnson PW, Kunze KL, van Helmond N, Klassen SA, Wiggins CC, et al. Program and patient characteristics for the United States Expanded Access Program to COVID-19 convalescent plasma. medRxiv. 2021:2021.04.08.21255115.

94 Casadevall A, Dragotakes Q, Johnson PW, Senefeld JW, Klassen SA, Wright RS, et al. Convalescent plasma use in the USA was inversely correlated with COVID-19 mortality. *Elife*. 2021;10.

95 Joyner MJ, Carter RE, Senefeld JW, Klassen SA, Mills JR, Johnson PW, et al. Convalescent plasma antibody levels and the risk of death from COVID-19. *N Engl J Med*. 2021;384(11):1015-27.

96 Agarwal A, Mukherjee A, Kumar G, Chatterjee P, Bhatnagar T, Malhotra P. Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial). *BMJ*. 2020;371:m3939.

97 Horby PW, Estcourt L, Peto L, Emberson JR, Staplin N, Spata E, et al. Convalescent plasma in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. medRxiv. 2021:2021.03.09.21252736.

98 Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. *JAMA*. 2020;324(5):460-70.

99 Libster R, Pérez Marc G, Wappner D, Coviello S, Bianchi A, Braem V, et al. Early high-titer plasma therapy to prevent severe COVID-19 in older adults. *N Engl J Med*. 2021;384(7):610-8.

100 Simonovich VA, Burgos Pratx LD, Scibona P, Beruto MV, Vallone MG, Vázquez C, et al. A randomized trial of convalescent plasma in COVID-19 severe pneumonia. *N Engl J Med*. 2020;384(7):619-29.

101 Senefeld JW, Klassen SA, Ford SK, Senese KA, Wiggins CC, Bostrom BC, et al. Use of convalescent plasma in COVID-19 patients with immunosuppression. *Transfusion*. 2021.

102 Klassen SA, Senefeld JW, Johnson PW, Carter RE, Wiggins CC, Shoham S, et al. The effect of convalescent plasma therapy on mortality among patients with COVID-19: systematic review and meta-analysis. *Mayo Clinic Proceedings*. 2021;96(5):1262-75.

103 Petkova E, Antman EM, Troxel AB. Pooling data from individual clinical trials in the COVID-19 era. *JAMA*. 2020;324(6):543-5.

104 Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He WT, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science*. 2020;369(6506):956-63.

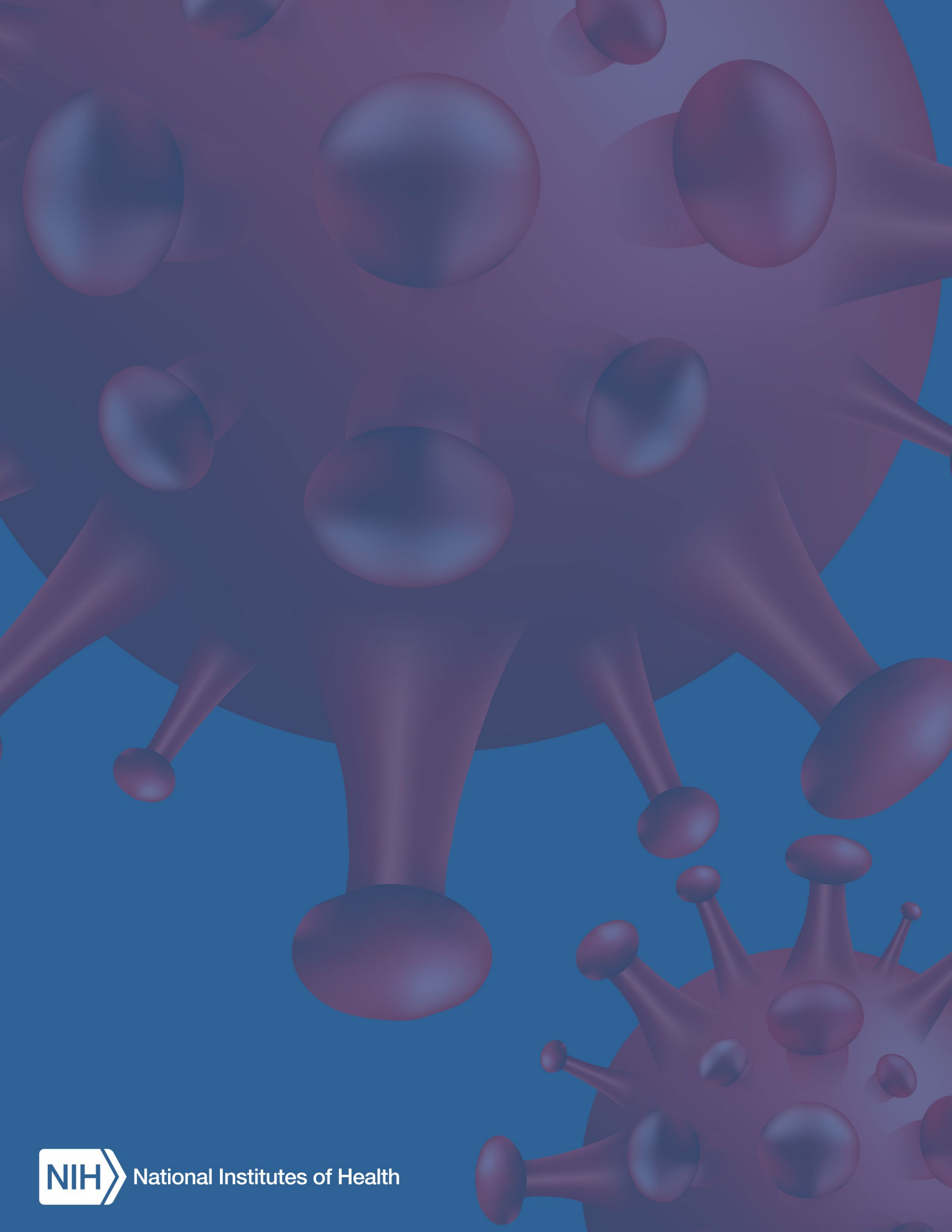
105 Group RC, Horby PW, Mafham M, Peto L, Campbell M, Pessoa-Amorim G, et al. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. medRxiv. 2021:2021.06.15.21258542.

106 U.S. Food and Drug Administration. https://www.fda.gov/drugs/coronavirus-covid-19-drugs/cder-scientific-review-documents-supporting-emergency-use-authorizations-drug-and-biological. Accessed on August 2, 2021.

107 Health Resources and Services Administration. https://coviduninsuredclaim.linkhealth.com. Accessed on August 5, 2021.

108 University of Pennsylvania Health System. https://microb120.med.upenn.edu/data/SARS-CoV-2. Accessed on August 5, 2021.

109 Bange EM, Han NA, Wileyto P, Kim JY, Gouma S, Robinson J, et al. CD8(+) T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med*. 2021;27(7):1280-9.



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