Therapeutics for COVID-19

Report from the isirv-AVG Virtual Conference, 6-8 October, 2020

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Abstract
The International Society for Influenza and other Respiratory Virus Diseases held a special Antiviral Group (ISIRV-AVG) virtual conference on 6-8 October 2020. The conference was an opportunity for investigators from academia, industry, and government to present and hear the latest pre-clinical and clinical advances on therapeutics against SARS-CoV-2 and COVID-19. The conference included discussions on the strategies for conducting clinical trials of therapeutics and perspectives for the clinical management of COVID-19 patients. The aim of this report is to summarize the main concepts and novel observations presented on therapeutics to make these available to the broader scientific community.

Keywords: COVID-19, SARS-CoV-2, antivirals, immunomodulators, clinical trials

Background

The Antiviral Group of the International Society for Influenza and Other Respiratory Virus Diseases (isirv-AVG) held the virtual conference on 6-8 October 2020, focused on the development of therapeutics for COVID-19 and other coronavirus infections. It was organized like prior isirv-AVG conferences with a mix of state-of-the-art talks, updates on candidate therapies in clinical development, abstract presentations on novel therapeutics, and a panel discussion on endpoints for clinical trials and regulatory issues. The state-of-the art presentations on COVID-19 clinical features (Cao Bin, China-Japan Friendship Hospital, Beijing), disease pathogenesis including viral replication kinetics and seroresponses (Malik Peiris, University of Hong Kong, Hong Kong), autopsy findings (Xiu-Wu Bian, Third Military Medical University, Chongqing, China), immunology (Peter Openshaw, Imperial College, London UK) and acute respiratory distress syndrome (Richard Wunderink, Northwestern University, Chicago, USA) and other presentations are available on the ISIRV website (https://isirv.org/site/index.php/avg-events). This report focuses on new observations regarding COVID-19 therapeutics, including the contributions of large platform clinical trials, presented during the conference. The rapid pace of research on the topics covered is reflected by the publication of the detailed findings of several clinical trials during and shortly following the conference [1–4].

1. Opening remarks

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During the last 20 years, we have experienced a series of emerging viral infections, such as the recurrent threat imposed by influenza but also including Nipah, SARS-CoV, MERS-CoV, Ebola, Zika, and the recently emerged SARS-CoV-2. In that regard, the role of environmental change and globalization on the risk and increased frequency of emerging infections cannot be underestimated. Many of these emerging viruses, including influenza, share roughly the same features: we have imperfect or no vaccines available, imperfect diagnostics, and a critical lack of effective treatments, treatments that can not only save the lives of those severely ill but also limit disease progression and transmission, hence reducing pressures on healthcare systems. And it is essential to make treatments and other science-based countermeasures accessible equitably for all people around the globe.

The COVID-19 pandemic is an opportunity to work together globally, share the responsibilities for gathering information and understanding SARS-CoV-2 infection and its differential consequences on the elderly and vulnerable risk groups, in order to transform our knowledge of an acute dynamic viral infection. While previous experience from other viral infections can be very valuable, it is important to see COVID-19 as unique, and not something that necessarily follows other emerging viral infections. Also, the scientific community needs to take a careful look at the design of randomized controlled trials (RCTs); many small studies have failed to be definitive because of their inadequate size and ambition. Indeed, by putting therapeutics into large scale pragmatic clinical trials such as SOLIDARITY and RECOVERY, we can get definitive answers on which therapeutics work and which do not.

Finally, the COVID-19 pandemic is a global challenge, and as such, having robust clinical research networks such as ISARIC, and the NIH networks like ACTT is critical. These networks cannot be established in the middle of a pandemic crisis, they have to be built, optimized, and remain active beforehand in order to unleash their full potential during the critical need phase of newly emergent pathogens, including a defined pipeline for the selection and evaluation of the full spectrum of innovative science-based therapeutic interventions. The input of regulators will be essential throughout the process to validate that the data generated are reliable and gathered correctly for subsequent authorization and licensing of emerging therapeutics.

2. Pre-clinical Studies of Direct Acting Antivirals for SARS-CoV-2

2.1 SARS-CoV-2 antiviral targets

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Compared to other respiratory RNA viruses, coronaviruses have a large genome of ~30kb. Interestingly, two-thirds of the genome encodes a multi-protein replicase complex of 16 non-structural proteins (nsp), including the nsp12 RNA-dependent RNA polymerase (RdRp) and the nsp14 exonuclease (ExoN) that are processed by viral proteases, notably the papain-like protease (PL^{pro}) and the 3 chymotrypsin-like protease ($3CL^{pro}$) [5]. All of these viral proteins and their interactions with one another or with host cell proteins are potential targets for novel antivirals.

Previous studies from Vanderbilt University found that inactivation of nsp14 resulted in a 20-fold increase in viral genome mutation rate, shedding light on a unique proof-reading function of this protein that facilitates high fidelity replication. While wild-type viruses with fully functional nsp14 were resistant to nucleoside analogues such as ribavirin or 5-flurouracil, nsp14

knock-down resulted in increased sensitivity to such drugs [6]. These results prompted the screening of other nucleoside analogs as potential antivirals with the goal of identifying ones with broad activity against coronaviruses, high barrier to resistance, extended therapeutic window for prevention, and potential for multiple modes of administration.
Remdesivir (or GS-5734) was developed by Gilead Sciences as a nucleoside inhibitor against Ebola virus. Following a series of *in vitro* screening experiments of different nucleoside analogs, remdesivir arose as a prodrug form of the monophosphate of GS-441524, a 1′-CN modified adenosine C-nucleoside hit. Remdesivir monophosphate is metabolized within the cells into its active nucleoside triphosphate derivative. In 2014, GS-5734 was found to have potent antiviral activity against coronaviruses. Indeed, GS-5734 induced a 6-log reduction in viral titers at a concentration lower than 1 µM, resulting in EC50 values in the nanomolar range. Although GS-5734 does not fully bypass the proof-reading function of nsp14, nsp14-defective viruses are more sensitive to GS-5734 [7]. The exact mechanism of antiviral action of remdesivir is yet to be determined, but studies suggest multiple mechanisms. Remdesivir has been shown to be a non-obligate chain terminator for first strand RNA synthesis and may also interfere with second strand synthesis [7–9]. Also, the antiviral effect of remdesivir may result from slowing down of the replicase activity [10].
Remdesivir shows antiviral activity against all bat and human respiratory coronaviruses tested to date. Of note, remdesivir has differential potency in different cell lines for SARS-CoV-2, with EC50 values of 1.65 µM in VeroE6 cells and 0.28 µM in Calu3 cells, suggesting varying cell-dependent capacity for drug penetration and phosphorylation. Remdesivir has also shown antiviral effectiveness in primary lung epithelial cells and in animal models [11–13]. Similar activity was observed for β-d-N4-hydroxycytidine (NHC), another nucleoside analog formerly named EIDD-2801 and now molnupiravir, although the mechanism of action differs from that of remdesivir. Although specific SARS-CoV-2 resistance selection studies are in progress, a previous study of serially passaged murine hepatitis virus (MHV) under remdesivir pressure enabled the selection of two point mutations in the nsp12 polymerase: F476L and V553L [7]. These two mutations induced a 6-fold increase in EC50 values, though the double mutant virus was outgrown by wild-type virus in *in vitro* competition experiments and showed reduced fitness and virulence in mice. Interestingly, mutant viruses with reduced susceptibility to remdesivir remain sensitive to NHC, opening perspectives for drug combinations between nucleoside analogs or with other classes of inhibitors.

2.2 Pre-clinical models for down-selecting candidates

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Broad-spectrum antivirals, as well as drug repurposing approaches and other therapeutic interventions (eg. convalescent plasma, monoclonal antibodies, immunomodulators) can play a particularly important role in the initial phases (first 12-18 months) of outbreak response and management, when neither vaccines nor specific antivirals against the newly emerging virus are available. In this context, pre-clinical experimental models of infection and treatment have proven essential for the initial screening and subsequent evaluation of candidates. Nonetheless, the pathway from pre-clinical testing to clinical validation of effective therapeutics has many pitfalls, some of which have been clearly highlighted during the COVID-19 pandemic.
In general terms, pre-clinical models can be classified in three categories: classic immortalized cultured cell lines, complex 3D *in vitro/ex vivo* cultures including organoids, and *in vivo* (animal) models. One comparative screen of SARS-CoV and SARS-CoV-2 replication in a broad range of animal and human-derived cell lines showed differences between the two viruses and highlighted Vero/VeroE6, Calu-3, Caco2 and Huh7 as cell lines supporting robust replication of SARS-CoV-2 [14]. A549 cells engineered to express the ACE2 receptor as well as

VeroE6 cells expressing the TMPRSS2 protease have also shown high permissiveness to SARS-CoV-2 infection [15]. Despite their usefulness for virus isolation and replication studies, the limited cell receptor repertoire and sometimes artificial infection or treatment scenarios favoring alternative viral entry pathways might bias interpretation of putative inhibitory effects of drug candidates, leading to false positive "hits". Indeed, despite showing EC50 values against SARS-CoV-2 in the low micromolar range in Vero cells, the putative inhibitory activity of chloroquine (CQ) and hydroxychloroquine (HCQ) was completely abrogated in both Calu-3 and TMPRSS2-engineered Vero cells [16]. In a poster presentation by Levi and colleagues, up to tenfold variation in IC50 values of sofosbuvir against SARS-CoV-2 depended on the cell line used (https://mod.ISIRV.org/repository/avg_2020/E-Poster_19.pdf).

Complex 3D *in vitro/ex vivo* cultures, notably reconstituted human airway epithelia (HAE) and organoids from primary human respiratory or pluripotent cells, represent a major step forward in terms of biological significance and predictive value. Although the use of HAE for the study of respiratory viral infections has been limited until recently, some key advantages compared to classic cell cultures (ie, human origin, full repertoire of respiratory cell receptors, (pseudo)stratified architecture, air-liquid interface, mucus secretion, cilia beating) and their immediate availability compared to SARS-CoV-2 small animal models have contributed to increased use during the pandemic. Reconstituted HAE enabled a thorough characterization of the main physiological features of SARS-CoV-2 infection in nasal, bronchial or alveolar respiratory tissues [11,17] and highlighted the much greater inhibitory effects of remdesivir in HAE compared to HCQ, both of which had shown comparable efficacy in conventional cell culture. These results were further confirmed in non-human primate (NHP) models [13,18]. Preliminary results on viral replication of SARS-CoV-2 in organoids were presented [19,20] but further data are warranted. Microfluidics-based organ-on-chip models [21] appear as interesting alternatives capable of integrating circulating immune cells, though the real potential of such models in the context of antiviral evaluation remains to be established.

Finally, animal models are the only pre-clinical models that allow, among other infection parameters, the assessment of treatment candidates against *in vivo* viral replication, clinical signs and symptoms, complex immune responses, viral transmission, and pharmacokinetic-pharmacodynamic relationships for these parameters. Animal models for SARS-CoV-2 infection and treatment evaluation (eg. HCQ, remdesivir, IFNs) have been thoroughly reviewed elsewhere [15,22,23]. These include wild-type mice infected with mouse-adapted SARS-CoV-2 viruses [24], mice permanently or transiently expressing genetically-modified or human ACE2 receptors [25], Syrian Golden hamsters [26], ferrets [27], and non-human primates [13,18]. Although no single model can mimic all parameters of SARS-CoV-2 infection in humans in terms of virus replication, clinical manifestations, drug pharmacology, transmission and immunology, non-human primates currently stand as the closest to mild-to-moderate human pathophysiology and are compliant with FDA approval. These models are limited in their need for BSL3 protection, which are not widely available.

In summary, pre-clinical models have played and will continue to play a significant role in the preliminary evaluation of COVID-19 therapeutics. Because there is no "one size fits all" solution for candidate downselection, an integrated approach combining the strengths and limits of different pre-clinical models is warranted. The validation of a potential candidate, ideally in two different cell lines, plus in a HAE-like model and finally in one or more animal models, should provide compelling evidence to support subsequent evaluation in clinical trials. This approach relies on harmonization of protocols and the establishment of networks and consortia that share their expertise on different models. Investment in high-quality, physiologically relevant and predictive pre-clinical evaluation strategies will improve overall efficiency and success rates in clinical development.

2.3 Repurposing of clinically-approved drugs for the treatment of COVID-19

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Based on an international collaborative proteomics study that generated a host cell-SARS-CoV-2 protein-protein interaction map [28], an academic-industrial partnership was established with the objective of identifying drug candidates with repurposing potential against SARS-CoV-2 based on their capacity to target key host factors involved in viral replication. After initial chemoinformatic screening and *in vitro* evaluation in classic cell models, the goal is to validate the inhibitory activity of candidates in animal models, as well as the putative mechanism of action and the potential for combination with remdesivir. Priority was given to drugs already approved or in clinical development to eventually facilitate their rapid transfer to clinical trials. Two classes of agents with antiviral activity in cell culture were of particular interest: inhibitors of mRNA translation and inhibitors of the regulation of Sigma-1 and Sigma-2 receptors at the endoplasmic reticulum. For example, oncologic drugs zotatafin, ternatin 4 and plitidepsin (Apilidin, PharmaMar) are inhibitors of the eukaryotic translation elongation factor 1 alpha 1 (cEF1A) at different stages of clinical development, which showed EC50 values against SARS-CoV-2 in cell culture of 101 nM, 1.6 nM and 0.7 nM, respectively. Prophylactic treatment of mice with 0.3 or 1 mg/kg Apilidin resulted in reduced SARS-CoV-2 viral replication on day 3 post-infection [29]. The clinical evaluation of Apilidin in hospitalized COVID-19 patients is currently ongoing (NCT04382066).

Alternatively, given the structural similarity between the hepatitis C virus (HCV) NS3/4A and the SARS-CoV-2 M^{Pro} viral proteases, docking studies identified inhibitors of the HCV protease, such as boceprevir and grazoprevir, that effectively inhibited SARS-CoV-2 viral replication in cell culture with EC50 values in the micromolar range. Interestingly, combination studies showed a 7-8 fold reduction in EC50 values of the grazoprevir plus remdesivir combination when compared to either drug alone, with a synergistic combination index of 0.45. Synergy was not observed for the boceprevir plus remdesivir combination.

2.4 Sirtuin inhibitors

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Sirtuins (SIRTs) are a family of seven cell proteins that regulate gene expression and cellular metabolism, including inflammation. Several sirtuins are nicotinamide adenine dinucleotide-dependent deacylases that are linked to the energy status of the cell, with the expression and function of SIRT2 being particularly induced during low-energy conditions [30]. Given the effect of viral infection on the ramp-up of host cell metabolism to provide building blocks for new virus particles, a potential role of sirtuin inhibitors as host-directed antivirals has been suggested. In that regard, SIRT2 inhibitors have been shown to reduce replication of various RNA and DNA viruses in pre-clinical models [31,32].

FLS-359 is a sirtuin inhibitor, designed by molecular docking analysis of the crystal structure of SIRT2, in order to block its peptide-binding channel and hence separate the acyl-lysine substrate from the NAD+ cofactor. FLS-359 is a dose-dependent inhibitor of SIRT2 and to lesser extent SIRT1 and SIRT3. Moreover, FLS-359 inhibited the replication of alpha (HCoV-H229E) and beta (HCoV-OC43) coronaviruses in MRC-5 cells at low micromolar concentrations. Time-of-addition experiments further demonstrated that FLS-359 reduced viral RNA levels even when added up to 8 h post-infection, which is consistent with the inhibition of a post-entry stage of the viral cycle. The effect of this host-directed inhibitor on the potential

induction of HCoV-OC43 drug resistant variants is currently under study. Interestingly, pre-treatment of Calu-3 cells with FLS-359 2 h before infection with SARS-CoV-2 resulted in a reduction of infected cells with an EC50 of 1.12 μM. The putative anti-SARS-CoV-2 activity of FLS-359 is currently being tested in an iPSC induced alveolar epithelial type II cell model. Animal model studies to assess the efficacy of FLS-359 against SARS-CoV-2 infection remain to be undertaken.

2.5 GC-376

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Proteolytic processing of the two major SARS-CoV-2 polyproteins ORF1a and ORF1b by the viral proteases PL^{pro} and $3CL^{pro}$ plays a central role in viral replication. Consequently, the identification and validation of viral protease inhibitors that stop the cleavage and activation of functional viral proteins required for replication stand out as an attractive antiviral strategy. In line with different drug repurposing approaches, previous molecular docking analyses highlighted GC-376, a potential inhibitor of $3CL^{pro}$ with potent pre-clinical activity against feline infectious peritonitis (corona) virus (FIPV), as a promising candidate against SARS-CoV-2 [33]. The inhibitory activity of GC-376 against SARS-CoV-2 was further validated in *in vitro* experiments using Vero cells [34,35].

In order to evaluate the putative antiviral activity of GC-376 against SARS-CoV-2 *in vivo* a transgenic K18-hACE2 mouse model was used [36], in which animals were randomized in 6 different groups, including two different virus inocula (10^3 or 10^5 TCID50/mouse). Treatment with 20 mg/kg BID of GC-376 was started shortly after viral infection and continued for 7 days. Animals were daily monitored for clinical signs such as weight change, physical appearance, and activity as well as mortality, until the end of the experimental protocol on day 14. No differences in the above mentioned parameters were observed between the vehicle and GC-376–treated, non-infected groups, indicating adequate tolerability under the conditions tested. Mortality differences were observed at the two different virus challenge doses tested, reaching 40% and 80-100% for the low and high inoculum doses, respectively. Treatment with GC-376 did not show significantly different outcomes compared to the vehicle-treated group regardless of the virus inoculum used, with comparable clinical presentation, weight loss and mortality. Studies of viral load quantification and immunohistochemical analysis of viral antigens and cellular immune markers are currently ongoing.

3. Clinical Trials of Direct Acting Antivirals for SARS-CoV-2

3.1 RECOVERY trial and strategies for rapid clinical testing

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Retrospective comparative analysis of data from the 2009 influenza pandemic between the expected enrollment of hospitalized or severe patients (~6000) and the final number of patients included in clinical trials with published results (~150) highlighted that very little reliable evidence was collected and also left the impression of having missed an opportunity for performing robust clinical trials. Once the COVID-19 outbreak was reported, the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) managed to rapidly plan and initiate RCTs in China for the evaluation of lopinavir/ritonavir and later remdesivir as therapeutic options, enabling rapid progress. Indeed, only 20 days passed from the official announcement of the outbreak to the enrollment of the first patient. However, the need to include more study sites in order to get a bigger sample size and hence robust results, combined

with the progressive reduction in the number of cases in China and the emergence of the epidemic in the UK, prompted a shift of RCTs to the UK.
The RECOVERY trial (NCT04381936) is the largest clinical trial for COVID-19 in the world. It was designed keeping in mind the unprecedented clinical challenge imposed by a potentially pandemic context, including an overstretched health service, pressure and stress of medical staff, large number of patients, and huge therapeutic uncertainty, notably, variable reliability of data on the various therapeutic candidates. As a result, the RECOVERY trial relied on three basic premises: be simple, be quick, and be big (to reach sufficient power to detect "moderate" benefits). In that regard, "being simple" included all materials being made available on-line and remote training of site staff: a simple consent form in multiple languages; simple web-based randomization; a simple follow up form with no specimens collected; and linkage to national data sources with permission to follow up via health records for up to 10 years. This approach enabled very fast implementation and scaling, with only 9 days between the first study protocol and the first patient enrollment, and 1,000 patients recruited in the first 15 days and 5,000 by day 28. This extraordinary timing and scaling were crucial for successfully catching most of the first COVID-19 wave in the UK. The current status of that trial shows that the first randomization managed to include ~13,200 patients, which enabled the gathering of robust data on the therapeutic potential of many interventions compared to the standard-of-care (SOC), including remdesivir, lopinavir/ritonavir, HCQ, corticosteroids, and azithromycin. A second randomization included approximately 1,000 additional patients for the evaluation of tocilizumab and approximately 1,000 patients for convalescent plasma (CP). Noteworthy, another arm aiming at evaluating the efficacy of Regeneron's REGN-COV2, an investigational anti-S monoclonal antibody cocktail, has been added to the trial.
Available results show that the mean age of recruited patients was 66 years (range, >1 to 102 years old). The mean time from inclusion to randomization through the electronic system was 6.75 days and median time was 5 days. Allocation to the HCQ group failed to reduce 28-day mortality and increased the duration of hospitalization, as well as the risk of progressing to mechanical ventilation or death compared to SOC [37]. Allocation to the lopinavir/ritonavir group showed no difference compared to SOC [38]. Importantly, allocation to the dexamethasone group resulted in one-third reduction of mortality in patients receiving invasive mechanical ventilation and one-fifth reduction in patients receiving oxygen without invasive mechanical ventilation, although no reduced mortality was observed in patients not receiving ventilatory support at randomization [3]. Recently, RECOVERY outcomes have reported no benefit from adding azithromycin [39] to SOC but that administering the IL-6 inhibitor tocilizumab further reduced mortality when added to dexamethasone [40].

3.2 Solidarity trial and lessons learned

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As an aftermath of the Ebola outbreak started in 2013 in West Africa, and at the request of its 194 Member States, the WHO created in May 2015 a broad network of experts to develop the R&D Blueprint, a global strategy and preparedness plan that would allow the rapid activation of R&D responses during epidemics. Its aim is to fast-track the availability of effective tests, vaccines and medicines that can be used to save lives and avert large scale crisis. Following the COVID-19 outbreak the R&D Blueprint rapidly created the Solidarity Trial (NCT04315948), a large open-label, pragmatic international clinical trial platform based on simple to collect endpoints in order to assess the efficacy of four therapeutics: remdesivir, HCQ, lopinavir/ritonavir, and the lopinavir/ritonavir + IFN-β1 combination. Patients are randomized equally to SOC alone or one of the intervention arms available locally. The primary endpoint of the trial is all-cause mortality, with secondary endpoints including duration of hospital stay

and time to mechanical ventilation or intensive care. Importantly, since realistic, appropriate sample sizes could not be estimated in the early days of the pandemic, the trial was conceived through an adaptive design with no pre-specified sample size but includes the possibility that reviews by the Global Data and Safety Monitoring Committee could decide to stop under-performing intervention arms as needed, eventually replacing them with new candidates to be evaluated. As in the case of the RECOVERY trial, eligibility (admitted or recently hospitalized adults) and consent forms were simplified and all materials were made available on-line, easing administrative burden and hence facilitating enrollment and follow up. Due to the ease of enrolling patients and collecting data, the study is currently being conducted in 500 hospitals in more than 30 countries globally, with 12,000 patients enrolled so far.

Important interim analysis of data from 405 hospitals in 30 countries has shown that of 11,266 randomized patients, 2750 were allocated to remdesivir, 954 to HCQ, 1411 to lopinavir/ritonavir, 651 to lopinavir/ritonavir + IFN-β1, 1412 to IFN-β1 only, and 4,088 to no study drug [4]. Compliance was 94-96% midway through treatment, with 2-6% crossover. Whereas 1253 deaths were reported (median day 8), Kaplan-Meier 28-day mortality was 12% (39% if already ventilated at randomization, 10% otherwise). The conclusion from these results is that the treatment regimens of remdesivir, HCQ, and lopinavir/ritonavir with or without IFN-β1 used in the study appeared to have little or no effect on hospitalized patients with COVID-19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay [4]. While further analysis is ongoing, investigators are interested in adding new treatment arms to the study, including antivirals, immunomodulators, and mAbs.

Finally, beyond the specific clinical results, both Solidarity and RECOVERY trials have arguably set new standards for robust clinical evaluation in epidemic or pandemic contexts, showing that the combination of established networks, old-fashioned randomization, and modern information technology can yield rapid and reliable therapeutic answers.

3.3 Remdesivir

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As mentioned above, remdesivir (GS-5734) is a nucleoside inhibitor with broad antiviral activity against filoviruses, paramyxoviruses and many bat and human coronaviruses. Readily available data and previous clinical evaluation of remdesivir for other viral infections made it possible to rapidly move this drug to the clinic early in the beginning of the pandemic, based on three key pieces of evidence: i) the already established preclinical profile, ii) existing human safety data in 500 patients from studies on Ebola, and iii) known scalable manufacturing capacity.

The first four published RCTs were designed to provide answers to two main questions, namely the safety and efficacy of remdesivir for the treatment of hospitalized COVID-19 patients, and the possibility of reducing the standard 10-day treatment duration [41–44]. Overall, results from these trials have provided some consistent data across studies. Patients treated with remdesivir have a shorter time to recovery, meaning shorter length of hospital stay and a shorter time to discharge. For example, in the ACTT-1 trial (n=1062) [41], time to recovery was shortened from 15 days in the placebo group to 10 days in the remdesivir group ($p<0.001$), as well as time to discharge or clinical score reduction (12 days vs 8 days) and duration of hospitalization (17 days vs 12 days). In the SIMPLE trial of moderately ill patients (n=584) [43] patients treated with remdesivir for 5 days were 65% more likely to show improved clinical status at day 11 compared to SOC ($p=0.02$), suggesting comparable efficacy between the 10- and 5-day treatment regimens. Both trials also showed reduced disease progression in patients treated with remdesivir, as measured by the ordinal clinical score and the need of oxygen supplement or

mechanical ventilation. However, no trial to date has definitely shown a benefit of remdesivir treatment in reducing mortality, which is further supported by recently published results of the ACTT-1 [41] and SOLIDARITY trials [4]. In that regard, initial RCTs were not designed to specifically measure the effect of treatment on overall mortality as a key outcome, and Gilead has a stratified post-hoc analysis to be published soon showing that treatment of patients early in the disease course (requiring low-flow oxygen supplementation) might result in improved survival. Noteworthy, no increased incidence of severe adverse events derived from remdesivir treatment when compared to placebo were reported in the ACTT-1 and SIMPLE Moderate studies.

Finally, preliminary data on the evaluation of remdesivir in children (n=77) and pregnant women (n=67) through Emergency Use Authorization of remdesivir showed that treatment was associated with clinical improvement at day 29 in both groups [45,46]. Gilead is currently investigating the use of remdesivir in combination with other potential COVID-19 treatments and looking to treat patients in the outpatient setting with an inhaled formulation.

3.4 Favipiravir

Yohei Doi, University of Pittsburgh, PA, USA

Favipiravir is a purine nucleoside analogue prodrug that undergoes intracellular ribosylation and phosphorylation to favipiravir triphosphate that acts as an inhibitor of many viral RNA dependent RNA polymerases, by its incorporation leading to chain termination and lethal mutagenesis. Its mechanism of antiviral action against coronaviruses remains to be fully established but appears to be similar [47]. The reported EC50 of favipiravir against SARS-CoV-2 have ranged from 9.7 to >78 μg/mL depending the cell type and assay utilized [48,49]. In Syrian hamsters infected with SARS-CoV-2, favipiravir pre-treatment (300-1,000 mg/kg/day) resulted in dose-dependent reductions in lung infectious virus titers, and high intraperitoneal doses improved lung histopathology and also reduced virus transmission when given to contact animals [26].

Professor Yohei Doi reviewed the findings of several recent favipiravir studies. An open-label RCT in 89 patients with asymptomatic or mild SARS-CoV-2 infection found no significant difference in early compared to delayed favipiravir administration in RNA negativity at day 6 (66.7% vs 56.1%), although the duration of fever tended to be about 1 day shorter in the early treatment group [50]. An open-label RCT enrolling 150 mild to moderately ill COVID-19 patients in India reported non-significant faster oropharyngeal viral RNA clearance and shortened time to clinical cure (median, 3 vs 5 days; HR 1.75 [95% CI 1.10, 2.79]; p=0.029) with favipiravir compared to control [51]. In a single-blind, placebo-controlled RCT of 156 Japanese patients hospitalized with COVID-19 pneumonia, favipiravir treatment (1,800 mg BID on day 1, followed by 800 mg BID from days 2 onwards) shortened the primary endpoint of time to alleviation of illness (based on temperature, oxygen saturation, and chest imaging) and RT-PCR negativity from 14.7 days in placebo to 11.9 days (aHR = 1.593; p=0.0136) [52]. In a small trial of 60 hospitalized COVID-19 in Russia, only one-quarter of whom required supplemental oxygen, treatment with favipiravir (1,800 mg BID followed by 800 mg BID or 1,600 mg BID followed by 600 mg BID) significantly increased viral RNA negativity at day 5 (62.5% vs 30.0%) and reduced fever duration (median, 2 vs 4 days) but not duration of hospitalization compared to SOC [53].

An observational study of 2,158 hospitalized patients in Japan, most of whom had mild or moderate COVID-19, reported clinical recovery in 74% by day 7 and 88% by day 14 [54]. At approximately 1 month the mortality rates were 5.1%, 12.7%, and 31.7% for those presenting with mild, moderate, and severe disease, respectively. However, whether early administration

of favipiravir might reduce the risk of developing critical illness and death remains to be determined.
Given the high concentrations needed to inhibit SARS-CoV-2 *in vitro* and the observation of unexpected low plasma levels in critically ill COVID-19 patients [55], much higher doses than those used in studies to date have been proposed for treatment of serious COVID-19 [56]. The most common adverse event with favipiravir is reversible hyperuricemia. Hepatic transaminase elevations rash may also occur, but no new safety signals have been detected in COVID-19 studies to date. Importantly, animal studies have demonstrated that the drug distributes to sperm and is teratogenic in multiple species, so that its use in pregnant women is contraindicated, and strict contraception should be enforced during and after its administration. Favipiravir is approved for treating COVID-19 or on compassionate or emergency use basis in several countries, and a new drug application is expected for this indication in Japan.

3.5 Molnupiravir (EIDD-2801/MK-4482)

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MK-4482, or molnupiravir, is an orally administered prodrug of the nucleoside analog ß-D-N4 hydroxycytidine (EIDD-1931). Once in the plasma MK-4482 is rapidly converted by esterases to EIDD-1931 that undergoes further intracellular metabolism to its triphosphate. EIDD-1931 can tautomerize to mimic both uridine and cytidine leading to accumulation of mutations that lead to noninfectious viral progeny. EIDD-1931 is an inhibitor of coronaviruses and many other RNA viruses including influenza, RSV, and Venezuelan equine encephalitis virus *in vitro* and in animal models [57–59]. The EC50 for SARS-CoV-2 is 0.3 µM in Vero cells, 0.08 µM in Calu-3 cells, and <0.1 µM in human airway epithelial cells [58]. In mice infected with SARS-CoV or MERS-CoV, both prophylactic and early therapeutic administration of oral EIDD-2801 improved pulmonary function and reduced lung virus titers and body weight loss [58]. A recent study showed positive effects of therapeutic treatment with EIDD-2801 in preventing SARS-CoV-2 transmission in ferrets [60].
The first in human study began in April 2020, and the phase 1 dose-ranging study found MK-4482 to be safe and generally well tolerated at all doses tested, including the maximum single dose of 1,600 mg and maximum multiple dose regimen of 800 mg BID for 5.5 days. The pharmacokinetics of EIDD-1931 were dose proportional with generally low inter-subject variability. Dosing with food resulted in a modest decrease in Cmax and prolongation of the plasma half-life but little change in AUC. The observed exposures are predicted to be in the therapeutic range for SARS-CoV-2 and influenza. Phase 2 studies with a range of dose regimens are currently in progress to assess viral clearance and safety endpoints in COVID-19 outpatients through the AGILE-ACCORD platform [61] and in hospitalized patients (NCT04405570; NCT04405739). A placebo-controlled, dose-ranging phase 2 RCT (200, 400, or 800 mg BID for 5 days) followed by a large phase 3 RCT assessing the selected dose in hospitalized COVID-19 patients enrolled within 10 days of illness onset, but excluding those with respiratory failure, is planned (NCT04575584). In parallel, similarly designed phase 2 followed by phase 3 RCTs are planned to be conducted in COVID-19 outpatients enrolled with 7 days of symptom onset with the primary endpoint being hospitalization or death (NCT04575597).

3.6 Sofosbuvir and Daclatasvir

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Sofosbuvir (SOF) is a polymerase inhibitor that interferes with the hepatitis C virus (HCV) NS5B protein, whereas daclatasvir (DCV) is an inhibitor of HCV NS5A, a non-structural protein involved in the replication complex. Whereas the SOF plus DCV combination has proved effective in the treatment of chronic hepatitis C, *in silico* analysis suggested that the combination might be a potential repurposed treatment against SARS-CoV-2 infection. *In vitro* data suggested that DCV could inhibit SARS-CoV-2 at micromolar concentrations, although the mechanism of action underlying this putative antiviral activity remains uncertain [62]. Although no evidence of anti- SARS-CoV-2 activity has been found for SOF in cell culture, it has been postulated to potentially enhance the effect of DCV. Moreover, results from a meta-analysis of three open-label studies in Iran reported faster recovery and reduced mortality in COVID-19 patients treated with SOF/DCV compared to control treatment [63]. However, the total sample size of the three trials combined was only 176 patients and one trial was not randomized.

A parallel 2-arm, open-label RCT in 89 adult COVID-19 inpatients in a single hospital in Cairo, Egypt (NCT04443725) randomized patients in the control arm (n=45) to receive only the SOC therapy according to the Egyptian Ministry of Health protocol, whereas the treatment arm (n=44) received SOC together with an oral daily dose of 400 mg SOF combined with 60 mg DCV from day 1 to 10. The primary endpoint was the proportion of clinical recovery (composite) within 21 days, defined as the normalization of fever (≤37.2 °C oral), respiratory rate (≤24/minute on room air), and oxygen saturation (≥94% on room air), and being sustained for at least 24 hours. The proportion of cumulative clinical recovery in the experimental group at day 21 was numerically greater than the control group (91% vs 77.8%), although the difference was not significant (RR: 1.17; CI: 0.97-1.4). Nevertheless, the Hazard Ratio (HR) for time to clinical recovery adjusted for baseline severity estimated by Cox-regression was statistically significant (HR: 1.59; CI: 1.001-2.5). This observation is in line with a non-statistically significant tendency of the experimental group towards improved lung lesion CT scores and lower overall case fatality rate (4.5% vs 11.1%) compared to the control group. On the other hand, no differences in virus clearance by day 21 were observed between the experimental and control arms (63.6% vs 60%, respectively). The results of a large Iranian double-blinded, placebo-controlled DISCOVERY trial comparing SOC with SOC plus SOF/DCV in more than 1000 COVID-19 patients are awaited with interest.

4. Interferons

In the context of COVID-19, SARS-CoV-2 has also been shown to modulate IFN-I and IFN-III responses in human respiratory cell lines, primary bronchial epithelial cells, and in the blood of hospitalized COVID-19 patients, with SARS-CoV-2 ORF6, ORF8 and nucleocapsid proteins identified as inhibitors of IFN-I signaling (Blanco-Melo et al., 2020; Li et al., 2020). Likewise, impaired IFN-I signatures, as compared with patients who have mild or moderate illness, inborn errors of IFN-I immunity, and auto-antibodies against IFN-α subtypes, and less commonly IFN-β, have been found in patients with critical COVID-19 [66–68]. One RCT in mild COVID-19 patients found that early treatment with a regimen of IFN-β1b given subcutaneously plus lopinavir-ritonavir and low-dose ribavirin alleviated symptoms and shortened the durations of viral RNA detection and hospital stay compared to lopinavir-ritonavir alone (Hung, 2020). Conversely, a preliminary report from the WHO Solidarity trial that initially randomized four different products (HCQ, IFN-β1a, lopinavir-ritonavir, remdesivir) and local standards of care

found that none of the treatment groups, including those given IFN-β1a (3 doses of 44 μg subcutaneous QOD or 10 μg intravenous once daily over six days) with lopinavir-ritonavir (651 patients) or IFN-β1a alone (1,412 patients), had significant reductions in mortality compared to control [4]. Also, none of the study drugs reduced initiation of ventilation in those who were not already ventilated. Several other IFN-β studies are ongoing, with one testing subcutaneous IFN-β1a aiming to enroll 1,000 COVID-19 patients (ACTT-3; NCT04492475). Of note, on September 29, 2020, the ACTT-3 trial was modified to stop enrolling severely ill COVID-19 patients requiring high-flow oxygen and not to begin enrolling patients requiring non-invasive or invasive mechanical ventilation [69]. The decisions were taken after an interim DSMB review found an imbalance of serious adverse events among patients on high-flow oxygen/non-invasive mechanical ventilation who received IFN-β1a versus those who did not. Of note, no safety concerns among study participants with less severe COVID-19 were raised. These findings are in line with previous data from animal models showing adverse effects of interferons later in coronavirus infections [70] and emphasizes the importance of early treatment.

4.1 Inhaled Interferon-β1a

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Interferons (IFNs) are a family of cytokines produced and released by host cells in response to infection, causing nearby cells to heighten their anti-viral defenses. Zoonotic coronavirues inhibit type 1 IFN production to evade host antiviral responses (Li et al., 2020) but are highly susceptible to inhibition in vitro by IFN-βs [71]. In the context of respiratory viral infections, one strategy to increase exogenous IFN effects in the respiratory tract is through topical administration. Studies at the University of Southampton have established that inhaled IFN-β is generally well-tolerated and induces antiviral biomarkers in sputum and blood cells [72]. Consequently, a randomized, double-blind, placebo-controlled trial was undertaken to determine the safety and efficacy of inhaled SNG001 (IFN-β1a for nebulization) for the treatment of adults with confirmed SARS-CoV-2 infection. The first 100 patients studied in the hospital setting had mild to moderate COVID-19 as reflected by a WHO Ordinal Scale for Clinical Improvement (OSCI) score of 3 or 4. Patients who were ventilated or in intensive care at baseline were excluded. The two groups (1:1 randomization) had experienced a median of 9.5-10 days of symptoms before enrollment. Six MIU of SNG001 or placebo was delivered using a mesh nebulizer once daily for up to 14 days, the primary endpoint being the change in ordinal scale score during the dosing period.

Initial analysis showed increased odds of improvement in OSCI scores for the SNG001 treated group compared to placebo (OR 2.32, 95% CI 1.07-5.04; p=0.033). The odds of developing severe disease, notably considering death and/or the need of ventilation, also tended in favor of SNG001 (OR 0.28, 95% CI 0.04-0.97; p=0.064). Moreover, SNG001 recipients were more than twice as likely to recover (OSCI score of 0 or 1) during the treatment period compared to placebo recipients (HR 2.19, 95% CI 1.03-4.69; p=0.043). SNG001 was associated with fewer serious treatment-emergent adverse effects, and no safety concerns were identified. Of note, three subjects (6%) died after being randomized to placebo, but no deaths occurred among patients treated with SNG001. No virology data were presented. In view of these results, an ongoing home-based study is aiming to enroll 120 patients who are earlier in the course of SARS-CoV-2 infection, and SNG001 is being considered as one arm of the RECOVERY trial.

4.2 Injected Interferon--β1b and lopinavir-ritonavir in MERS

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Middle East Respiratory Syndrome Coronavirus (MERS-CoV) first emerged in 2012 and still circulates, particularly in the Middle East, with approximately 2500 cases reported in 27 countries and 858 deaths, resulting in a case fatality ratio (CFR) of 34%. MERS-CoV causes severe ARDS with multi-organ involvement, and in the Kingdom of Saudi Arabia, which accounts for 85% of total MERS-CoV cases reported so far, the mortality in critically ill MERS patients has been significantly higher than that found in non-MERS critical acute respiratory illness (67% and 35%, respectively) [73]; the CFR of COVID-19 among ICU patients in the same hospital setting is about 25%. In terms of pathogenesis, while MERS-CoV infection inhibits type I IFN (IFN-I) responses, it is also inhibited by IFN-I, especially IFN-β *in vitro* and in animal models [74,75]. In a murine model of MERS-CoV infection, blocking IFN-I signaling delayed virus clearance and impaired T cell responses. Moreover, IFN-β administration within 1 day after infection (before peak virus replication) protected mice from lethal infection, whereas delayed IFN-β treatment failed to inhibit virus replication, increased infiltration of activated inflammatory cells, enhanced pro-inflammatory cytokine expression, and worsened outcomes [76]. While MERS-CoV appears to be more sensitive to IFN effects than SARS-CoV, no comparative studies using SARS-CoV-2 are currently available.

The MIRACLE trial is the first double-blind, placebo-controlled RCT of antiviral therapeutics in MERS and investigated the efficacy of a combination of recombinant IFN-β1b and lopinavir/ritonavir compared to placebo on 90-day all-cause mortality in hospitalized patients with laboratory-confirmed MERS-CoV [1]. In part because of the uncertain effect of size due to the limited number of MERS-CoV cases, the study used an adaptive two-stage recursive design that allowed for adjustment of the trial parameters using data observed during prior stages without inflation of the type I error, as contrasted to a less-powered classic two-study approach with a pilot study followed by the main trial [77]. Adults with confirmed MERS-CoV infection and new organ dysfunction judged to be related to MERS were randomized to a regimen of 0.25 mg (8 million IU) IFN-β1b by subcutaneous injection on alternate days plus 400/100 mg oral lopinavir-ritonavir twice daily for up to 14 days intervention or placebo groups. Because of the COVID-19 pandemic, the Data and Safety Monitoring Board (DSMB) held an unplanned interim analysis on March 15, 2020 and recommended early termination of the trial, at which point 95 patients (43 intervention, 52 placebo) had completed follow-up. Randomization was stratified by receipt of invasive mechanical ventilation (40% intervention, 42% placebo) or not, and the majority of enrolled patients were in intensive care at enrollment. Taking account of the adaptive design of the study, the primary outcome measure, namely 90-day mortality was significantly lower in the intervention group compared to placebo (29% and 48%, respectively, p=0.024). In 49 patients starting treatment within 7 days from symptom onset, mortality was reduced from 46% to 9% (p=0.006), but no benefit was observed with later treatment. Median time free from invasive or non-invasive mechanical ventilation were numerically higher in the intervention compared to placebo group (16 and 5.5 days, respectively). No apparent effect on viral RNA clearance in respiratory samples was found. Importantly, serious adverse events, primarily transaminase elevations, were more common in the placebo group (19.2%) than intervention (9.3%) group. In conclusion, the MIRACLE trial showed that treatment with recombinant IFN-β1b and lopinavir/ritonavir resulted in lower 90-day mortality than placebo in hospitalized adults with MERS-CoV, specifically in those treated within 7 days after symptom onset.

5. Antibody-based Therapeutics

5.1 Convalescent plasma and polyclonal antibodies

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The rationale for the use of convalescent plasma (CP) relies on the principle of passive immunity, namely transferring Abs generated from a recovering patient (or animal) to prevent or treat disease in another patient. Historically, this approach was successfully used for the treatment of patients during the 1918 influenza pandemic, being reported to alleviate fever in a few days [78]. A meta-analysis [79] showed that mortality was more than halved in patients receiving CP compared to those who did not receive treatment (16% vs 37%, respectively). A RCT in patients with Argentine hemorrhagic fever [80] also showed significant reduction of case-fatality rates among cases treated with CP (1.1%) compared to controls treated with normal plasma (16.5%). One observational study during the SARS-CoV epidemic found that relatively early use of CP before day 14 and before development of seropositivity was associated with significantly greater proportions with day 22 hospital discharge [81].

In the context of the COVID-19 pandemic, a US-based network of experts in the use of CP self-assembled in March and rapidly drafted protocols for prophylaxis and then for early treatment of hospitalized patients. National interest and an FDA decision to grant emergency compassionate use through an investigational new drug (IND) process increased demand for CP and rapidly prompted the development of an expanded access program (EAP). On 23 August, the FDA issued an Emergency Use Authorization, and enrollment was stopped in the EAP. From April to August, there were more than 78,774 patients transfused in 2,800 hospitals across the USA. As regards safety, there were very low rates for transfusion-associated circulatory overload (TACO, 0.14%) and transfusion-related acute lung injury (TRALI, 0.22%) in critically ill patients [82]. Severe allergic transfusion reactions were estimated at 0.06% of recipients. Caution is required with regard to efficacy analysis, because CP use was not randomized, the CP was uncharacterized with respect to neutralizing Ab content or other immune activity, variable volumes were given to patients, and the patient population was heterogeneous. Nevertheless, potential signals of efficacy were found. For example, patients receiving two units of CP showed a slightly lower crude 30-day mortality than those who received one unit (22.4% vs 25.4%, respectively). Early transfusion (given within 5 days from diagnosis) seemed to confer better protection than late transfusion (6 days or later). A subset analysis found that death within 30 days after CP transfusion occurred in 22.3%, 27.4%, and 29.6% of recipients in the high, medium, and low titer anti-SARS-CoV-2 IgG groups, respectively, although no benefit was found in those receiving mechanical ventilation [83]. Importantly, no antibody-dependent enhancement (ADE) of disease was observed in transfused cases. Interim analysis of the EAP data indicates that further validation of CP for treatment of COVID-19 should be based on early treatment with sufficient does of high neutralizing Ab titers. Of note, recent RCTs of CP in hospitalized COVID-19 patients in India, Argentina, and the UK have reported disappointing results with no reductions in mortality [84–86], and most COVID-19 patients already have high neutralizing antibody titers at hospital admission [87]. Another trial found that early administration (within 3 days of symptom onset) of high-titer CP in high-risk outpatients found reduced risk of disease progression [88], and other studies are in progress .

5.2 Monoclonal antibodies

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Despite the effort and investment put into the development of COVID-19 vaccines to protect individuals and control the evolution of the pandemic, wide-scale immunization with effective and safe vaccines will take considerable time. In addition, some will not get vaccinated, either because they do not want to or cannot for medical reasons and others will not develop robust and/or lasting immunity. One major goal of vaccination is to generate neutralizing Abs, which can alternatively be delivered passively as a treatment for infected individuals at high risk of disease progression or prophylactically to health care workers or specific high-risk groups. The challenge underlying this approach is that there are millions of possible antibodies in polyclonal sera, which raises many practical questions; how to choose the best Abs? What makes them the best and which assays can be used to assess them? If Ab cocktails may help to mitigate escape, what would be the best pairing?

In this regard, the international and multi-disciplinary Coronavirus Immunity Consortium (CoVIC) group of experts from academic and industry settings aim to advance effective, Ab-based therapies against SARS-CoV-2. The focus is to evaluate the landscape of monoclonal Ab candidates currently being developed across multiple labs through independent, standardized platforms, and to identify a cocktail of human neutralizing mAbs against the spike protein to prevent severe COVID-19. Ab candidates that could be developed rather inexpensively and hence be widely available in low- and middle-income countries are a top priority. This initiative will also provide valuable insight on the scientific aspects of Ab landscape and their activity, Ab features that correlate with protection, and on which current assays (and animal models) best correlate with success in humans. The need for this type of technical knowledge stems from a lesson learned from the VIC consortium working on Ebola virus; Abs that neutralized Ebola virus *in vitro* did not protect NHP from infection, though a cocktail of thee non-neutralizing Abs protected NHP from lethal challenge [89]. Indeed, the VIC consortium blindly compared 168 different mAbs to Ebola virus and found there is a complex and puzzling conundrum between neutralization and protection *in vivo*; some Abs neutralize and protect, some neutralize but not protect, and some do not neutralize but will protect. When comparing those Abs that neutralize but do not protect in animal models with those that do not neutralize but protect, it stood out that protection seemed to be strongly linked to the capacity of the Ab to recruit Fc-mediated effector cells, notably those with phagocytic and natural killer activity. As a result, it was possible to identify and categorize a set of 17 different parameters that can be combined through weighted analysis to predict the protective capacity of an Ab *in vivo*.

In practice, CoVIC follows the same general process developed for VIC, by putting Abs against the SARS-CoV-2 spike protein into a blinded process for systematic structural and functional analysis by 107 collaborating groups, in order to downselect candidates heading to clinical trials. Importantly, blinding and data sharing are central features of the program. CoVIC principal investigators and all reference labs are blinded to mAb name and source, funding program officers and the CoVIC program manager are unblinded but keep data confidential, and contributors only know the code names of their own mAbs so they can view data as it is collected and eventually request re-analysis if data is not as expected. Contributors also retain all industrial property rights on their own mAbs and may publish and develop as they wish.

5.3 SARS-CoV-2 spike receptor-binding domain escape variants

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The receptor-binding domain (RBD) of the SARS-CoV-2 Spike protein plays a central role in viral infection through the binding to ACE2 on target cells to mediate virus entry. As a result, the RBD is a target of choice for neutralizing Ab capable of blocking its ability to interact with ACE2. However, amino acid substitutions at the RBD could affect Ab recognition and hence have important implications for both Ab-based therapeutics and vaccines. But how can we measure the effects of all potential substitutions in the RBD and anticipate their potential impact on the design and evaluation of RBD-directed interventions? A yeast display-based approach was designed in order to enable high-throughput Ab binding experiments. This strategy relies on the expression of a library of mutant fluorescent-tagged RBDs on the surface of yeast cells, with every cell carrying one different variant. The fluorescent tag enables measurement of the RBD expression level and binding to specific ligands, notably ACE2 and Abs [90]. Briefly, the yeast library of RBD variants is incubated with a panel of Abs directed to the RBD, and flow cytometry analysis is used to sort out the cell populations with reduced binding to Ab ("antibody escaped" population). Then, comparative deep sequencing is performed between the initial library ("pre-selection" population) containing the complete repertoire of mutants and the "antibody escaped" mutant population in order to quantify escape. Results are expressed as logo plots, in which at every single position of the RBD the size of the amino acid letter is proportional to the impact of that specific mutation on reducing Ab binding. This deep mutational strategy was successfully applied to produce high-resolution antibody escape maps to 10 human mAbs: 9 neutralizing Abs isolated from SARS-CoV-2 convalescent patients, plus a recombinant form of one cross-reactive non-neutralizing Ab isolated from a convalescent SARS-CoV patient [91]. While many Ab-selected escape mutations are within the receptor-binding motif and near the ACE2-contact site, they all harbored their own unique sets of escape substitutions, named "escape maps".

In parallel, a VSV pseudovirus system expressing the SARS-CoV-2 spike protein was used to perform virus growth under pressure from two of the Abs for which escape maps were previously obtained (COV2-2050 and COV2-2499). Ab pressure prompted the emergence of some major predicted RBD escape mutations (E484K for COV2-2050, G446D and Q498R for COV2-2499) in up to 13% and 31% of the replicates, respectively. Further analysis revealed that not only their single-nucleotide nature but also the negligible negative impact on ACE2 binding argue in favor of the selection of these particular mutations among the different potential escape variants predicted at those specific sequence positions. Interestingly, although an escape map was predicted for the COV2-2165 Ab, *in vitro* experiments failed to select any escape mutants after 56 replicates. This observation is consistent with the fact that most of the single-nucleotide predicted substitutions show a significantly deleterious effect on either ACE2 binding or RBD expression. Finally, the combination of COV2-2050 and COV2-2499, two antibodies that have different escape mutation profiles despite competing for binding, also failed to select any escape mutants in 80 replicates, paving the way for considering this mutation scanning approach for the rational design of antibody cocktails [91]. Rapid escape from individual anti-RBD directed mAbs and the ability of non-competing mAb combinations to reduce this risk have been reported by other groups [92].

6. Host-directed therapeutics with antiviral effects

6.1 SLV213, a novel cysteine protease inhibitor

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Upon binding of SARS-CoV-2 to ACE2 receptors on the cell surface, the activation of the spike protein needed for viral entry can be mediated by two host proteases: the Cathepsin L cysteine protease or the TMPRSS2 serine protease. *In vitro* experiments have shown that blocking TMPRSS2 can partially reduce viral entry but blocking Cathepsin L can completely block the process. The cysteine protease inhibitor SLV213 has been in development during recent years as an anti-parasitic drug against Chagas disease. Previous studies reported that besides its capacity to block the Cruzain protease of *T Cruzi*, SLV213 is a potent dose-dependent inhibitor of Cathepsin L activity [93,94]. *In vitro* cytopathic effect-based experiments using VeroE6, Calu-3 and ACE2-expressing A549 cells showed that the addition of SLV213 concomitantly with SARS-CoV-2 inhibited viral infection, with EC50 values, respectively, of 0.62, 5, and <0.08 µM [95]. Treatment combination experiments with SLV213 and the TMPRSS2 inhibitor camostat are currently ongoing.

In a pilot NHP (African Green Monkey) study, animals were pre-treated with 100 mg/kg of SLV213 (four treated and two placebo) and then inoculated 3-4 hours later with SARS-CoV-2. Oral treatment was administered daily for 7 days, after which animals were euthanized, with viral load and organ function/pathology to be determined. Gross pathology data showed an increase in the weight of the lungs in the placebo group, which was not observed in the treated group (unpublished data from Selva Therapeutics). This observation is in line with previous reports in NHPs treated with remdesivir [13]. Histopathology data shows a protection of lung tissue from diffuse alveolar damage, which was observed in all control animals and is found in COVID-19 patients with acute respiratory distress syndrome. Finally, a recent Phase I RCT conducted in healthy volunteers met its primary objective of demonstrating safety and tolerability of SLV213 [96], for which phase II clinical trials are expected to start soon.

7. Host immunomodulatory therapeutics

7.1 Fluvoxamine

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Extensive reports indicate that COVID-19 patients can show significant clinical deterioration during the second week of illness, usually due to an excessive inflammatory response with associated hypercytokinemia. This clinical finding supports considering potential therapeutic approaches not focused on reducing viral infection itself but mainly on modulating host pro-inflammatory immune responses. Selective serotonin reuptake inhibitors (SSRIs) have varying actions at the Sigma1 receptor (S1R) chaperone protein of the endoplasmic reticulum (ER) that interacts with the inositol-requiring enzyme 1α (IRE1) stress sensor. This stress response of the ER is involved in both virus-host interactions and regulation of cytokine production, for which it represents an interesting therapeutic target for COVID-19.

The S1R agonist fluvoxamine has been previously shown to prevent death in a murine model of inflammation and sepsis and to reduce cytokine production in human blood exposed to LPS [97]. Based on these data, the STOP COVID clinical trial (NCT04342663) was set up with the objective to determine whether fluvoxamine, given during mild COVID-19 illness, prevents clinical deterioration and decreases the severity of disease. In this double-blind, randomized, placebo-controlled, fully remote (contactless) trial, adult outpatients with confirmed SARS-

CoV-2 infection and showing symptoms for <7 days were randomized in two arms, receiving either placebo (n=72) or 100 mg fluvoxamine (n=80) three times daily for 15 days. The primary outcome was clinical deterioration within 15 days of randomization defined by two parameters: shortness of breath or hospitalization for shortness of breath or pneumonia, and oxygen saturation <92% or need for supplemental oxygen.
Of the 152 patients that completed the trial, 0/80 in the treatment group met the primary endpoint of clinical deterioration, compared to 6/72 (8.3%) in the placebo group (p=0.009). The rate of reported serious adverse events during the 15-day trial was lower in the fluvoxamine group. One participant in the fluvoxamine group had a serious adverse event, while 5 participants in the placebo group had at least one serious adverse event [2]. No cases of respiratory deterioration occurred during a 30-day follow-up; thus, fluvoxamine appeared to prevent rather than delay deterioration. A large placebo-controlled, blinded RCT enrolling approximately 1,100 COVID-19 outpatients is currently in progress to confirm the effects of fluvoxamine (NCT04668950).

7.2 Janus kinase (JAK) inhibitor treatment in mice

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Janus tyrosine kinase (JAK) family of proteins have been identified as crucial proteins in signal transduction initiated by a wide range of membrane receptors. Among the proteins in this family JAK1 and JAK2 have been associated with important downstream proteins, including signal transducers and activators of transcription (STATs), which in turn regulate the expression of a variety of proteins involved in induction or prevention of apoptosis [98]. JAK inhibitors are potent immunosuppressive agents by interfering with phosphorylation of STATs. Given their proven efficacy against diseases with excessive cytokine release such as rheumatoid arthritis, psoriatic arthritis, or ulcerative colitis, JAK inhibitors have emerged as potential therapeutic candidates against COVID-19.
Baricitinib (trade name Olumiant, Eli Lilly and Company) is an oral inhibitor of JAK1 and JAK2 that is approved for the treatment of active rheumatoid arthritis in adults in many countries, and of particular interest for COVID-19 treatment because of its documented anti-inflammatory properties and potential inhibition of coronavirus replication mediated by its affinity for AP2-associated protein kinase 1 (AAK1), leading to reduced SARS-CoV-2 endocytosis. An antiviral effect of baricitinib has been reported in liver organoids at high concentrations [99].
However, because type I IFNs trigger the JAK/STAT signaling pathway responsible for the activation of many antiviral genes upon viral infection, it is arguably possible that an inhibitor like baricitinib could facilitate virus replication. Baricitinib treatment reduced immune activation in SARS-CoV-2 infected rhesus macaques, including lower alveolar macrophage cytokine and chemokine responses, decreased infiltration of neutrophils into the lung, and limited lung pathology [100]. This immunomodulatory effect did not affect viral RNA loads nor did it adversely diminish type I IFN responses. On the other hand, treatment with baricitinib or tofacitinib (another FDA-approved oral JAK inhibitor) showed a quite different outcome when evaluated in a murine model of SARS-CoV infection with a virulent mouse-adapted strain. Prophylactic and therapeutic treatment with three different doses of baricitinib (3, 10, and 30 mg/kg BID) and Tofacitinib (5, 15, and 50 mg/kg BID) were evaluated, with each lowest dose corresponding to the therapeutic equivalent used in humans. Regardless of treatment initiation (prophylactic or therapeutic), baricitinib monotherapy caused significant dose-related increases in mortality, lung injury and pulmonary viral loads, with positive correlations being observed between viral loads and lung pathology. A similar profile was observed with tofacitinib monotherapy but only at high doses. The mechanism underlying these observations was likely

related to blockade of type 1 and 2 IFN antiviral responses, although further studies to confirm or infirm this hypothesis are in progress. These findings argue for study of JAK inhibitors in context of antiviral therapy to mitigate adverse effects on viral replication.

7.3 Janus kinase (JAK) inhibitors in humans

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Small observational studies of baricitinib in patients hospitalized with COVID-19 pneumonia have reported clinical benefits from adding baricitinib to standard of care [99,101,102]. The NIAID sponsored a randomized, placebo-controlled clinical trial (ACTT-2) evaluating the safety and efficacy of a combined remdesivir plus baricitinib treatment for hospitalized COVID-19 patients compared to remdesivir alone [103]. The primary endpoint of time to recovery (ordinal scale 1-3) was reduced by one day from median of 8 days (IQR 7, 9) to 7 days (6, 8) in the combination groups (OR 1.1.6, p=0.04). The subgroup of patients receiving high flow oxygen (group 6) appeared to experience the greatest clinical benefit (median time to recover, 10 days vs 18 days), whereas those not requiring supplemental oxygen had no apparent benefit. Overall, mortality was numerically lower in the combination group (5.1%) compared to remdesivir alone (7.8%). Baricitinib recipients had fewer SAES (16.0% vs 21.0%), less use of systemic corticosteroids, but possibly increased risk of thromboembolism. A recent observational study in hospitalized COVID-19 reported reduced mortality in baricitinib recipients [104]. Other clinical studies, including the COV-BARRIER trial (NCT04421027) that is studying baricitinib as a monotherapy and in the RECOVERY platform, are in progress. Of note, baricitinib has received emergency use authorization by the FDA for combined therapy with remdesivir in COVID-19 patients with severe or critical disease [105].

7.4 Selinexor for severe COVID-19

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Selinexor (XPOVIO®) is a selective inhibitor of nuclear export currently approved for the treatment of multiple myeloma and lymphoma. It binds to exportin 1 (XPO1), which has recently been described as a mediator in SARS-CoV-2 replication, also having a central role in inflammation through the regulation of NF-kβ and COX-2 pathways [106]. Following the hypothesis of potential dual anti-viral and anti-inflammatory effects in SARS-CoV-2 infection, including the direct interference with the nuclear export of critical viral and host proteins, pre-clinical studies were performed. Both prophylactic and therapeutic treatment with 10-100 nM selinexor inhibited SARS-CoV-2 *in vitro*, also inducing a significant nuclear accumulation of the ACE2 receptor (unpublished data from Karyopharm Therapeutics). Preliminary *in vivo* studies also showed that selinexor decreased pulmonary viral load, as well as rhinitis and alveolitis in infected animals.

XPORT-CoV-1001 (NCT04349098) was an international, phase 2, randomized, single-blind study to evaluate the activity and safety of low dose oral selinexor (KPT-330) in hospitalized patients with severe COVID-19. Patients having at least one classic symptom of acute respiratory infection and at least one sign of lower respiratory disease, were randomized in two groups, receiving standard of care (SOC) plus either oral placebo or oral selinexor 20 mg on days 1, 3 and 5 of each week for up to two weeks (the same 60 mg/week dose used to treat lymphoma). The primary endpoint of the trial was the proportion of patients with at least 2-point improvement in the clinical ordinal scale from baseline to day 14, and secondary endpoints including overall death rate on day 28, proportion of patients needing mechanical ventilation, time to mechanical ventilation, and time to a 2-point improvement on the ordinal scale. Among 117 patients enrolled, the primary and secondary clinical endpoints were not met

and outcomes were comparable between the selinexinor and placebo groups. Yet, conversion to negative SARS-CoV-2 RT-PCR was observed for 36.4% of treated patients compared to 19.6% in the placebo group. Time to RT-PCR negativity was also shorter in the treated group. Preliminary subgroup (n=66) analysis of patients with low LDH (≤370 U/L) or low D-dimer (≤600 mcg/L FEU) levels showed higher hospital discharge by day 14 (78.9% vs 57.1%), higher rate of patients meeting 2-point ordinal scale improvement (78.9% vs 64.3%), higher conversion to negative PCR (42.1% vs 28.6%), and reduced cytokine levels at day 8 in the treated group compared to placebo, respectively. This trend was not observed in the high LDH or high D-dimer subgroup. Future studies to validate these results as well as to evaluate the safety and efficacy of oral selinexor in outpatients or hospitalized patients with mild/moderate COVID-19 are warranted.

7.5 Leronlimab for mild to moderate COVID-19

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Cytokines, chemokines, and their receptors play a critical role in the recruitment, activation, and coordination of leukocytes in the pathophysiology of lung inflammation. Hypercytokinemia, also called "cytokine storm", is a physiological reaction in which the innate immune system causes an uncontrolled and excessive release of pro-inflammatory cytokines/ chemokines that is believed to play an integral role in the development of acute respiratory distress syndrome (ARDS). In the specific case of COVID-19, ARDS seems to be the result of the accumulation of neutrophils within the pulmonary circulation and alveolar spaces [107]. Leronlimab (PRO 140) is a humanized IgG4κ monoclonal antibody to the C-C chemokine receptor type 5 (CCR5) that inhibits the migration of Tregs into areas of inflammation. This inhibition might downregulate the innate immune response against pathogens and, most importantly, the migration of macrophages and release of pro-inflammatory cytokines in lungs (eg. IL-1β and IL-6), hence potentially mitigating the cytokine storm. Given the fact that CCL5 (RANTES), the ligand of CCR5, is secreted not only by T cells but also by respiratory epithelial cells upon binding of SARS-CoV-2, leronlimab has been considered as a potential therapeutic candidate for the treatment of severe COVID-19. In that regard, 65 patients with severe and critical COVID-19 infection (>50% intubated at baseline) have been treated with 700 mg Leronlimab under individual patient emergency use IND authorization, the majority of them showing improved or sustained clinical outcome (unpublished data from CytoDyn Inc).
In addition, two randomized controlled trials, CD10_COVID19 (NCT04343651) and CD12_COVID19 (NCT04347239) are evaluating leronlimab for the treatment of COVID-19 in mildly-to-moderately or severely ill patients, respectively. While the CD12_COVID19 phase 2b/3 study is currently ongoing, the CD10_COVID19 phase 2 study has been recently completed. This phase 2, two-arm, randomized, double-blind, placebo-controlled multicenter study evaluated the safety and efficacy of leronlimab in outpatients with mild-to-moderate COVID-19. Patients were randomized to receive weekly subcutaneous doses of 700 mg leronlimab (56 patients), or placebo (28 patients), for a total of 2 doses. Primary outcome measures were defined as an improvement in total symptom score for fever, myalgia, dyspnea and cough on day 14. Main secondary outcome measures included time to clinical resolution (TTCR) by day 14, change from baseline in National Early Warning Score 2 (NEWS2) and pulse oxygen saturation (SpO2) on days 3, 7, and 14. Baseline demographics were comparable between the two groups, with a median age of 55 years and 60% being female. Baseline characteristics in terms of age group (<60 vs ≥60 years), total symptom score group (≤4 vs >4), use of off-label COVID-19 treatments (eg. HCQ, CQ, azithromycin, levofloxacin, ceftriaxone, piperacillin/tazobactam), and consumption of tobacco products were also comparable.

In patients with Total Symptom Score ≥4 at baseline (higher score indicating a worse health state), 90% of subjects treated with leronlimab reported improvement in the total clinical symptom score compared to 71% of subjects in the placebo group on day 3. When all treated patients were considered (mITT population), the proportions were 63% and 56% for the treated and placebo groups, respectively. Although no differences in oxygen use or hospitalization rates were observed, 50% of patients in the treated group experienced improved NEWS2 scores on day 14 compared to 21% of patients receiving placebo (p=0.0223), suggesting that leronlimab could moderate illness progression. Of note, the study was limited by its exclusion of patients with pre-existing medical conditions including severe pulmonary, liver, and renal disease, who have been shown to be at higher risk for COVID-19 progression and overall mortality. Results of the CD12_COVID19 phase 2b/3 study are expected by April 2021.

7.6 Lanadelumab

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Activation of the host kallikrein-kinin system (KKS) has been postulated as a potential contributing factor for the development of ARDS [108], particularly considering the contribution of plasma kallikrein to different drivers of ARDS in COVID-19 patients. Hence, it could be expected that the modulation of the KKS may improve or prevent respiratory deterioration in affected patients. In that regard, a recent case-control study indicated that icatibant, an antagonist of the bradykinin 2 receptor, improved oxygenation of COVID-19 hospitalized, non-intubated patients (n=9) compared to matched controls [109].

To study this compound, a phase 1b double-blind study (NCT04460105) aiming at evaluating the safety and tolerability of the recombinant human monoclonal antibody lanadelumab, a specific inhibitor of plasma kallikrein currently approved for the treatment of angioedema, in adult patients hospitalized with COVID-19-related pneumonia. Key inclusion criteria are: hospitalization with RT-PCR-documented SARS-CoV-2 infection, evidence of COVID-19 pneumonia, and peripheral oxygen saturation ≤93% or respiratory rate ≥30 breaths per minute. Among others, use of immunomodulators, bradykinin or PK inhibitors within 3 months of screening is a key exclusion criteria. The study includes a single and a repeat-dose cohorts of 12 subjects each, randomized 3:1 to receive SOC plus placebo (n=3) or SOC plus 300 mg of intravenous lanadelumab (n=9) on day 1 or on days 1 and 4 for the single or multiple dose groups, respectively. Primary endpoints include treatment-associated adverse events and their severity, adverse events of special interest, clinical laboratory test results, vital signs including ECG, and physical examination. Secondary endpoints are mostly focused on pharmacokinetics and pharmacodynamics of lanadelumab and their relationship with patient exposure and response to COVID-19. This trial should provide valuable data on the utility of PK inhibition in COVID-19 and on the safety and tolerability of lanadelumab on this patient population. Noteworthy, the study was closed out in early 2021 and lanadelumab is being now investigated in a phase 3 study along with additional candidates (NCT04590586).

7.7 Tocilizumab

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Early data from patients with severe COVID-19 have shown higher IL-6 serum levels when compared to patients with mild or moderate disease, in line with the hypothesis of hypercytokinemia playing a key role in disease severity. Tocilizumab is an IL-6 receptor blocking antibody, approved by the FDA for treatment of the cytokine release syndrome (CRS) that may occur following some forms of immunotherapy [110]. Pre-clinical results showing a beneficial role of tocilizumab in different models of influenza infection or sepsis as well as

some observational studies [111,112] have served as a rationale to consider the potential utility of tocilizumab in the context of severe COVID-19.
The safety and efficacy of tocilizumab for the treatment of hospitalized subjects with COVID-19 was investigated in the COVACTA trial (NCT04320615), a randomized placebo-controlled trial of 450 patients. Key eligibility criteria were PCR-confirmed SARS-CoV-2 infection with signs of pneumonia and oxygen saturation ≤93%. Subjects were randomized 2:1 to receive SOC plus 8 mg/kg tocilizumab (up to 800 mg) intravenously, or SOC only. There was an optional second dose of tocilizumab if no clinical improvement was observed. The primary endpoint was clinical status at day 28 using a 7-category ordinal scale. The primary endpoint was not met for the study, with an odds ratio of 1.19 favoring tocilizumab but the 95% confidence interval spanning 1.0 (0.81 - 1.76) [113]. Trends were observed in some of the secondary endpoints for tocilizumab-treated patients compared to placebo, including a decreased time to discharge (20 vs 28 days, p=0.0370) and decreased risk of clinical failure (29.0% vs 42.2%, p=0.0253), defined as new mechanical ventilation, ICU transfer, or death. However, although a reduced duration of ICU stay was observed (9.8 vs 15.5 days, p=0.0370), tocilizumab treatment failed to reduce mortality. In line with that, recently published results from a RCT (n=243, NCT04356937) assessing the efficacy of tocilizumab in moderately ill hospitalized patients with COVID-19 found that treatment was not effective for preventing intubation or death [114]. Overall, the incidence of severe adverse events (SAEs) was low in both treatment and control arms but importantly, no increased incidence of infections was seen in the tocilizumab treated arm.
The EMPACTA trial (NCT04372186) is similar to COVACTA but focused on less severely ill hospitalized patients (patients under non-invasive or mechanical ventilation were excluded). As recently announced, it did meet its primary endpoint of significantly reducing clinical failure (defined as the risk of mechanical ventilation or death) in tocilizumab-treated patients (HR= 0.56 [0.32-0.97], p=0.0348) [115]. The REMDACTA trial (NCT04409262), a study with a more targeted enrollment and also evaluating the tocilizumab plus remdesivir combination is currently ongoing. In addition, the RECOVERY trial in hospitalized patients [40] and ReMapCap trial in critically ill ICU patients [116] have reported improved outcomes including mortality reductions with tocilizumab use.

8. Clinical management and perspectives

8.1 Operation Warp Speed therapeutics development

Janet Woodcock, CDER, FDA, Washington DC, USA

Operation Warp Speed (OWS) is a joint effort of the US Department of Health and Human Services and Department of Defense, started around 15 May 2020 to do everything possible to make highly performing, thoroughly evaluated vaccines, drugs, and diagnostics available in the US as soon as possible and ideally during the 2020 calendar year. The basic approach is to offer financial, manufacturing, logistical, scientific, medical, and regulatory assistance to leading candidates. The criteria for candidate therapeutic selection include scientific merit with strong mechanistic rationale, successful animal model studies or early clinical signals, manufacturability and feasibility to make products at commercial scale by at the least 1st quarter of 2021. This largely means repurposed drugs with antiviral potential (eg, remdesivir) and certain SARS-CoV-2-specific products (eg, monoclonal or polyclonal neutralizing antibodies) but not de novo small molecule development. Efforts on therapeutics have encompassed a rapid, comprehensive inventory of ongoing development programs worldwide and ongoing prioritization assessments based on scientific merit and manufacturing for leading candidates. Following identification of lead candidates and assessment of developmental needs,

teams have been created to work closely with selected manufacturers. Some (large) companies need no assistance but seek advance purchasing agreements with various conditions in the event their product should prove successful. Other companies need little clinical assistance but require funding for at risk manufacturing scale-up or with logistical help with supply chain bottlenecks, but many companies need assistance in conducting clinical studies.
The clinical programs of OWS are linked to another public-private partnership initiative, Advancing COVID-19 Therapeutics and Vaccines (ACTIV), launched led by Dr. Francis Collins in April 2020 and run by the Foundation for the NIH. ACTIV has performed rapid surveys of potential clinical trial networks, screened many compounds for scientific merit, developed criteria for candidates to enter clinical trials, and selected three immunomodulators to test (ACTIV-1), and developed two antiviral treatment "Master Protocols", one for outpatients (ACTIV-2) and one for hospitalized patients (ACTIV-3) that launched in August. Of note, the Lilly neutralizing anti-RBD monoclonal antibody, banlamivimab, has been tested in both trials, but was not effective when added to remdesivir in treating hospitalized COVID-19 patients compared to remdesivir alone [117]. ACTIV-4 is performing three separate studies of anticoagulation in outpatients, hospitalized patients, and discharged patients). OWS is also supporting non-ACTIV trials of hyperimmune globulin and convalescent plasma.
Clinical evaluation of candidate therapeutics has moved more slowly than hoped. Many challenges exist for conducting clinical trials with COVID19 patients, particularly competition for enrollment of patients and having adequate staff dedicated to research activities. There are a large number of ongoing studies, although many are underpowered or observational in design, and competing platform trials and industry-sponsored trials in the US. Prompt enrollment of infected outpatients has also been problematic, in part because existing clinical trial networks are based in academic medical centers and not in community sites which have most of the patients. Also certain study interventions, like monoclonal antibodies given by infusion, are difficult in an outpatient setting.

8.2 Discussion on clinical trial endpoints

Moderated by Michael Ison, Northwestern University, Evanston, IL, USA & Marco Cavaleri, European Medicines Agency, Amsterdam, Netherlands

Defining the optimal endpoint for clinical trials of respiratory viral infections has been an ongoing challenge. Most studies have focused on otherwise healthy ambulatory patients and utilized improvement of clinical signs and symptoms of infection as their primary endpoints. Unfortunately, these clinical endpoints have not been able to be used in studies of hospitalized patients with respiratory viral infections [118]. Since currently, endpoints have to measure how patients feel, function or survive, an array of endpoints have been utilized in studies of influenza and RSV antivirals (**Table 1**). An interagency working group of government and non-government experts focused on defining potential endpoints for inpatient studies of influenza antivirals [119]. The work of this group refined the ordinal scale and demonstrated its ability to correlate with clinically relevant differences in outcomes. The group also identified the National Early Warning Score (NEWS) as a potential way to risk stratify patients on enrollment. The Ordinal Scale was embraced by the WHO as a potential endpoint for clinical trials and has been used to identify effective therapies for COVID-19 [41,103]. Alternative endpoints that have been utilized in COVID-19 trials include viral load changes, duration of clinical signs and symptoms and need for hospitalization or supplemental oxygen among ambulatory studies, and mortality and duration of hospitalization among trials of hospitalized patients [3,39,120,121].

As part of the panel discussion, the experts discussed a range of other critical issues related to endpoints. One key point of discussion was that there is likely a different set of endpoints

needed for early therapies and antivirals compared to agents designed for later course therapy including immunomodulators. Biomarkers have been much discussed but, to date, have not been leveraged to inform optimal timing of specific interventions. Likewise, given that COVID-19 symptoms may persist over time, the impact of antivirals and immune modulators on these persistent symptoms and return to normal function. Areas of controversy that require additional data is whether adding other adverse outcomes, including renal dysfunction, liver dysfunction and heart failure, to hypoxemia in the ordinal scale. It was noted that there is very limited data from completed clinical trials on virology and particularly resistance emergence. Lastly, there was discussion of the need to convene expert groups to refine and outline data needed to inform consensus endpoints for future clinical trials.

8.3 WHO update on COVID-19 clinical management

Janet Diaz, WHO Health Emergency Program / Health Care Readiness Unit, *Geneva, Switzerland*

Multiple consequences and uncertainties encountered during this pandemic have adversely impacted clinical management of infected patients. Health care worker infections, in part due to inadequate access to personal protective equipment, have depleted the work force. The scarcity of other resources, like rapid diagnostics, hospital and intensive care beds, ventilators, and trained staff, have contributed to suboptimal care. The lack of known effective therapeutics has led to many small, non-definitive studies of repurposed drugs, uncertainty about their effectiveness because of the lack of clinical trials, and reliance on indirect evidence to guide supportive care interventions based on evidence for other causes of sepsis or respiratory failure. Confusion, rumors, and misleading information on appropriate care and treatments have been problematic since the inception of the pandemic.

The WHO has catalyzed an ongoing series of postings on clinical management that are evidenced-based, multidisciplinary, and focus on doing the basics well (https://www.who.int/teams/health-care-readiness-clinical-unit/covid-19). The basics cover screening and triage, clinical assessment (eg, risk factors, disease severity); and supportive care including oxygen therapy (on the WHO Essential Medicine List), advanced respiratory support strategies (eg, high-flow nasal oxygen [HFNO], non-invasive [NIV] or invasive mechanical ventilation [IMV]), and evaluation and management of complications (eg, sepsis, neurologic, cardiac, thrombotic). With regard to therapeutics, WHO has provided guidance on antivirals (new or repurposed), immunomodulators, and other interventions (eg, therapeutic dose anticoagulants, convalescent plasma) that should not to be used for treatment or prophylaxis outside of clinical trials. The guidance has undergone frequent revisions and the plan is to extract the therapeutics into a "living guidance" that can be updated quickly as new clinical trial data become available. In addition to fostering research like the Solidarity platform trial, WHO has embraced a 5-step approach to improve the efficiency of evidence generation and monitoring, evidence synthesis with the support of on-call methodologic teams, and formulating guidance with transparency and trustworthiness through standing guideline development groups and external reviewers (stakeholders, implementors). This process led to rapid guidance, recommending use of corticosteroids in severely or critically ill COVID-19 patients and recently against the use of intravenous remdesivir in hospitalized COVID-19 patients, principally based on the findings in the Solidarity trial [4]. The latter is a controversial recommendation as multiple other expert groups (eg, NIH, IDSA, SCCM) have promulgated their own guidelines and recommend early remdesivir use in hospitalized patients.

Because respiratory interventions are also a priority, with particular focus on low and middle income countries, WHO is assisting in developing operational research protocols, cohort studies, and eventually a RCT to test interventions, such as HFNO, NIV, awake prone in

intervention trial, to see if need for IMV is indeed reduced. WHO has also implemented a clinical data platform because clinical characterization is a priority in parts of the world where disease is less well described and patterns may differ, and because better understanding of mid-long term outcomes (eg, post-COVID syndrome, multisystem inflammatory syndrome in adults, pregnancy impacts) is needed.
Dr. Diaz concluded that the pathway of evidence generation to synthesis to guidance and ultimately to patient care requires a global perspective necessary to set priority research agenda, solidarity in its implementation, and collaborative in order to save lives all over the world.

9. Conclusions

As highlighted in the first section of the conference, the complex immunopathology induced by SARS-CoV-2 infection and the variable, multi-dimensional clinical features of COVID-19 represent considerable challenges for the identification and implementation of effective therapeutics, notably in the urgent context imposed by the pandemic. While clinical trials have shown little benefit from virus-directed repurposed drugs (eg, hydroxychloroquine, lopinavir), promising antiviral approaches are in different stages of clinical development, ranging from broader spectrum direct-acting antivirals, like polymerase inhibitors (eg. remdesivir, molnupiravir, favipiravir) to SARS-CoV-2 specific monoclonal and polyclonal antibodies. Further pre-clinical work is progressing on the development of SARS-CoV-2 selective direct acting and host-directed antivirals. The results of clinical trials have clearly shown that immunomodulatory interventions, like dexamethasone, the JAK inhibitor baricitinib, and the IL-6 receptor blocker tocilizumab, can mitigate host pro-inflammatory responses to infection and may improve clinical outcomes in some categories of hospitalized COVID-19 patients. Limited findings suggest that immunomodulatory interventions may be beneficial in outpatients, although data are less robust than in sicker patients. However, there is a consensus on the fact that no “one size fits all” therapeutic solution will be possible, for which many promising candidates might find their niche. To that end, it is of utmost importance to continuously improve pre-clinical and clinical experimental designs and data reporting and to rely on large-scale pragmatic clinical trials such as SOLIDARITY and RECOVERY in order to get definitive answers on which therapeutics work and which do not. Further, markers need to be identified to better tailor application of therapies for treatment of SARS-CoV-2.

Declarations of interests

AP is co-founder of Signia Therapeutics SAS, a spinoff of Université Claude Bernard Lyon 1 dedicated to drug repurposing against respiratory infections. MGI received research support, paid to Northwestern University, from AiCuris, Janssen and Shire; he is a paid consultant for Adagio, AlloVir, Celltrion, Cidara, Genentech, Roche, Janssen, Shionogi, Viracor Eurofins; he is also a paid member of DSMBs from Janssen, Merck, SAB Biotherapeutics, Sequiris, Takeda and Vitaeris. FGH serves on the DSMB for the phase 3 trial of leronlimab in hospitalized COVID patients and has been an unpaid consultant to multiple companies developing COVID-19 therapeutics or vaccines including Arcturus, Cidara, Fujifilm, Gilead, GSK, Merck, Pardes Bio, Ridgeback, Roche, Takeda, Vir.

Disclaimer

Readers are encouraged to read the primary peer-reviewed literature for more details about the studies and topics presented.

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Table 1. Endpoints Utilized for Studies of Hospitalized Influenza and RSV

Drug	**Sponsor**	**Status**	**Outcome measure**
Baloxivir	Roche	Completed	Time to Clinical Improvement (Hospital Discharge OR NEWS2 ≤ 2 for 24 hours)
Danarixin	GSK	Completed	Time to Clinical Resolution (discharge or temp, O2 sat, and 2 of 3 (RR, HR, SBP))
IVIG	NIAID	Completed	Day 7 Ordinal scale
Oseltamivir	NIAID	Completed	% negative viral RNA day 5
Peramivir	Biocryst	Completed	Time to Clinical Resolution (4 of 5)
Peramivir	Biocryst	Completed	Change viral titer in 48 h
Peramivir	Biocryst	Completed	Time to Clinical Resolution (4 of 5)
Peramivir	CUHK	Completed	change in influenza RNA load
Pimodivir	Janssen	Suspended	Day 6 Ordinal scale -Hospital Recovery Scale
Plasma	NIAID	Completed	Day 7 Ordinal scale
Plasma	NIAID	Completed	Time to Normalization of Respiratory Status (hypoxia and tachypnea)
Vis410	Visterra	Completed	Day 7 Ordinal scale
Zanamivir	GSK	Completed	Time to Clinical Resolution: (4 of the 5 vital signs (temp, O2 sat, RR, HR, SBP) or hospital discharge)
Presatovir	Gilead	Completed	Time-Weighted Average Change in Respiratory Syncytial Viral (RSV) Load From Baseline to Day 5