

SARS-COV-2 ANTIVIRAL THERAPEUTICS SUMMIT REPORT

Summit sponsored by the National Institute of Allergy and Infectious Diseases
and the National Center for Advancing Translational Sciences

NOVEMBER 6, 2020



Summit Report Authors

Annaliesa Anderson, Pfizer

James M. Anderson, Office of the Director, NIH

Kara Carter, Evotec*

Tomas Cihlar, Gilead Sciences

Anthony J. Conley, National Institute of Allergy and Infectious Diseases, NIH

Mindy I. Davis, National Institute of Allergy and Infectious Diseases, NIH

Mark Denison, Vanderbilt University

Matthew D. Hall, National Center for Advancing Translational Sciences, NIH

Daria Hazuda, Merck & Co.

Stephanie Moore, University of Alabama at Birmingham

George Painter, Emory University

Pei-Yong Shi, University of Texas Medical Branch

Richard Whitley, University of Alabama at Birmingham

**Current affiliation: Dewpoint Therapeutics*

Summit Hosts

Dr. Francis Collins, Director, National Institutes of Health (NIH)

Dr. Anthony Fauci, Director, National Institute of Allergy and Infectious Diseases (NIAID)

Dr. Christopher Austin, Director, National Center for Advancing Translational Sciences (NCATS)

Summit Organizers

James M. Anderson, Office of the Director, NIH

Kyle R. Brimacombe, NCATS, NIH

Anthony J. Conley, NIAID, NIH

Mindy I. Davis, NIAID, NIH

Stephanie Ford-Scheimer, NCATS, NIH

Abigail Grossman, NCATS, NIH

Matthew D. Hall, NCATS, NIH

TABLE OF CONTENTS

Introduction	5
Overview of the Virus and Therapeutics Approaches	7
Viral Replication Machinery	11
Proteases (Viral and Host)	18
Emerging Targets, Emerging Modalities	25
Preclinical Tools	28
Lessons from Other Viruses and Preparation for the Future	33
Summary of Discussions and Perspectives on the Challenges Ahead	38
Resources	43
References	47

INTRODUCTION

NIH SARS-COV-2 ANTIVIRAL THERAPEUTICS SUMMIT

Presenters:

Dr. Francis Collins (NIH)

Dr. Anthony Fauci (NIAID)

Dr. Christopher Austin (NCATS)

INTRODUCTION

The NIH Virtual SARS-CoV-2 Antiviral Summit was held on November 6, 2020. The virtual Summit was organized to provide an overview on the status and remaining challenges in developing antiviral therapeutics for COVID-19, including combinations of antivirals, and streamed live to allow broad public access while maintaining social distancing. Scientific experts from the public and private sectors were brought together to discuss SARS-CoV-2 targets for drug development, and the preclinical tools needed to evaluate and develop effective small molecule antivirals. The goal of the Summit was to review the current state of the science, identify unmet research needs, share insights and lessons learned from treating other infectious diseases, identify opportunities for public-private partnerships, and assist the research community in designing and developing antiviral therapeutics.

The Summit was jointly organized by NIAID, NCATS, and the NIH Office of the Director (NIH OD), and hosted by the respective Directors: Dr. Francis Collins (NIH), Dr. Anthony Fauci (NIAID), and Dr. Christopher Austin (NCATS). The meeting moderators and panelists were from academia, industry (pharma/biotech), NIH Institutes and Centers, and Federal agencies working in the COVID-19 therapeutics space.

The Summit itself was comprised of introductory remarks, an overview of the virus and therapeutic opportunities, followed by five scientific panels. In each panel, the session moderator provided an overview of the topic (slides are included in the Appendix) and facilitated discussion with panelists. An update on vaccines and neutralizing antibodies was provided, and a final summary session was held to identify key points from the Summit. The sessions were:

- Viral Replication Machinery
- Proteases (Viral and Host)
- Emerging Targets, Emerging Modalities
- Preclinical Tools
- Lessons from Other Viruses and Preparation for the Future

This Summit report is structured to reflect the meeting itself, providing an overview of the virus and therapeutics approaches, individual panel summaries, and a summary of the discussions and perspectives on the challenges ahead. The report was written utilizing the meeting transcript and recorded notes, and it was prepared by the Summit organizers with contributions and input from session moderators and panelists.

OVERVIEW OF THE VIRUS AND THERAPEUTICS APPROACHES

Speaker:

Dr. Mark Denison, Vanderbilt University

OVERVIEW

Coronaviruses are enveloped positive-sense single-stranded RNA viruses enclosed by capsid comprised of multiple proteins, most notably the spike proteins that are responsible for the virus's crown-like appearance. Cellular entry of coronaviruses, such as SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus-2), can occur in a number of ways, primarily via the spike (S) proteins of SARS-CoV-2 binding to the host ACE2 (Angiotensin-converting enzyme 2) receptor¹. Two-thirds of the SARS-CoV-2 genome is dedicated to the synthesis of two replicase polyproteins called polyprotein 1a and polyprotein 1ab. Within the polyprotein are two proteases, the papain-like protease (PL^{pro}) and 3CL^{pro} (also known as nsp5 or M^{pro}, short for main protease). In general, any inactivation of these proteases leads to a loss of RNA synthesis, and it is well established that disruption of viral RNA replication or viral protease functions are vulnerable to intervention^{2,3}. The next step after the formation of replicase proteins is the modification of host cell membranes, probably occurring in parallel to replication processes. A multiprotein complex formed by coronaviruses termed the replication transcription complex (RTC) is responsible for RNA replication and proof-reading. Nonstructural proteins nsp7 through nsp16 form the core of the RTC and represent a prime target for antiviral drug development, as the function of the core replicase is highly conserved. A detailed outline of the viral replication process, and druggable targets, is displayed in Figure 1.

Importantly in the context of antiviral drugs, when the SARS-CoV-2 epidemic (now a pandemic) emerged, there were no approved treatments or vaccines for treating any betacoronavirus infection. The endemic common cold betacoronaviruses OC42-CoV and HKU1-CoV generally cause mild symptoms, and have not stimulated significant therapeutic or vaccine development investigation. The SARS (caused by SARS-CoV-1) and MERS (Middle East Respiratory Syndrome, caused by MERS-CoV) epidemics were resolved using public health measures, though the translational science response to the SARS-CoV-2 pandemic has benefited from the intensive research efforts that took place to understand the SARS and MERS viruses^{4,5}.

From a therapeutic perspective, there are multiple windows of opportunity for preventing and managing COVID-19, which include the prophylactic pre-infection stage, early post-infection pre- or asymptomatic stage (antiviral window), and the symptomatic stages during which antiviral efficacy wanes and treatment strategy shifts towards immunomodulatory and anticoagulant therapies. As antiviral drugs are developed, some principles for SARS-CoV-2 antiviral development to be considered include:

- Antivirals should be broadly active for coronaviruses in both in vitro and in vivo models.
- Bioavailability of antivirals should be sought for multiple routes of administration: IV, oral, inhalational, intranasal, etc.

OVERVIEW

- Antivirals should have limited or no toxicity, especially for outpatient use, have a high barrier to resistance, and a large therapeutic window.
- Combinations of antivirals should be a priority in order to maximize potency and prevent resistance emergence. Combinations of antivirals and immunomodulatory therapies will be needed for treatment in later stages of illness.
- Vulnerable populations should be considered when designing new therapeutics as well as designing clinical trials.

Antiviral drug development for SARS-CoV-2 should take place with the recognition that unlike SARS and MERS (that sporadically re-emerges), the pandemic may continue for a long time, and the virus itself will likely be with us forever (endemic). There is also the high likelihood for another novel coronavirus(-es) to emerge from animal reservoirs. Given these realities, irrespective of vaccine effectiveness, there will be a need for antivirals. Teams working together across industry, academia, and government can drive fundamental discovery for this virus, and create the “playbook” for responding to the next zoonotic coronavirus. In the context of future pandemics, antivirals with broad-spectrum activity against betacoronaviruses would be positioned to enable rapid pre-clinical testing and clinical trials if and when a new zoonotic coronavirus appears (and for sporadic MERS outbreaks)⁶.

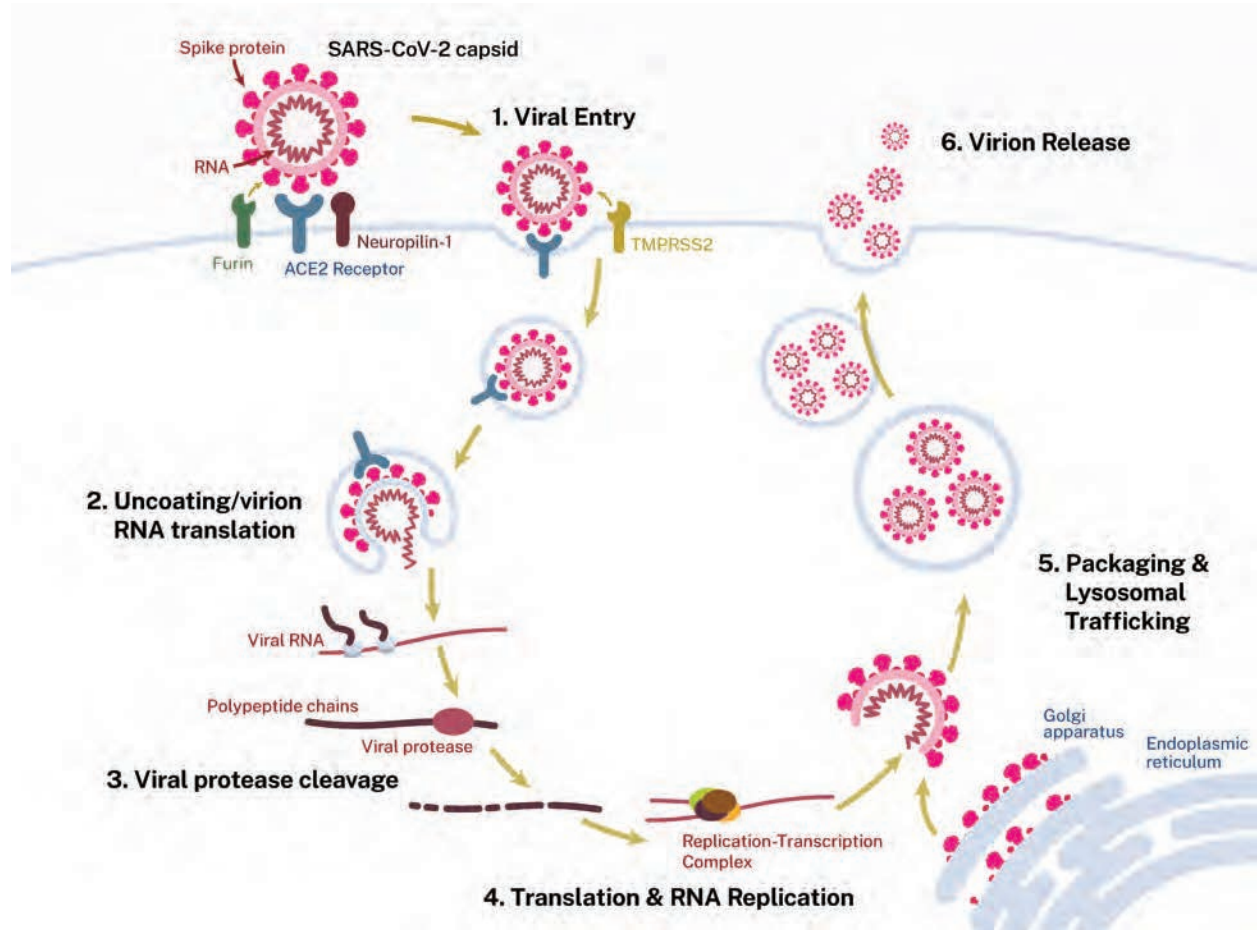


Figure 1. Scheme showing the SARS-CoV-2 viral replication cycle and highlighting “druggable” events. The SARS-CoV-2 virion is composed of a capsid protein coat, with an internal core of the viral genetic material (RNA). **1. Viral entry.** The SARS-CoV-2 virion in the extracellular space presents Spike (S) protein on the capsid surface. The S protein contains a number of protease cleavage sites, as well as the receptor binding domain (RBD). Engagement of spike protein at the extracellular surface involves engagement of multiple host cell proteins including (but may not be limited to) cleavage of S by the protease furin (gene FURIN), binding of the S RBD to ACE2 (ACE2), engagement of a liberated S terminal peptide to neuropilin-1 (Nrp-1, NRPI), and cleavage of S by the serine protease TMPRSS2 (TMPRSS2). **2.** Following endocytosis, the virion is uncoated, and the viral RNA translated into polypeptide chains. **3.** Two **viral proteases** cleave the viral polypeptide chains to produce up to 29 mature protein products. These proteases are the main protease (M^{pro}) and the papain-like protease (PL^{pro}). **4. Viral RNA replication** follows, with the formation of a replication-transcription complex, incorporating the RNA-dependent RNA polymerase (RdRp). **5.** The arising viral RNA (genetic material) is then packaged into capsid formed by viral protein including envelope (E), membrane (M), nucleocapsid (N) and aforementioned spike. Mature packaged virion is then trafficked via lysosomes, and **6. virion is released** via exocytosis. For other viruses, the time from viral entry to emergence of first progeny (the “eclipse time”) can be as little as 15 minute or as long as 7-8 hours⁷. Schematic prepared by Kyle R. Brimacombe (NCATS).

SESSION 1

VIRAL REPLICATION MACHINERY

Moderator:

Dr. Tomas Cihlar, Gilead Sciences

Panelists:

Dr. Elizabeth Campbell, Rockefeller University

Dr. Matthias Götte, University of Alberta

Dr. George Painter, Emory University

Dr. Michael Sofia, Arbutus Biopharma, Inc.

Dr. Sandra Weller, University of Connecticut

Summary of Current Status

Since the emergence of SARS-CoV-2 at the beginning of 2020, considerable progress over a very short period of time has been made in the development of antivirals for the treatment of COVID-19, in part because of ongoing studies for inhibitors of MERS-CoV. The identified agents include small molecule antivirals and biologics targeting both viral and host functions critical for SARS-CoV-2 infection and replication. Multiple potent neutralizing antibodies targeting independent epitopes on viral spike protein alongside a battery of direct acting small molecule antivirals interfering with various steps of viral replication have been quickly identified and brought to clinical testing. Across these efforts viral RNA synthesis stands out as one of the most critical functions for selective targeting by antiviral therapies⁸.

SARS-CoV-2 and other coronaviruses contain a ~30kb positive-sense non-segmented single strand RNA genome. Their RNA synthesis is a complex process consisting of two independent parts:

1. Viral genomic RNA replication through synthesis of a full-length negative (-) strand copy of the viral RNA genome that serves as a template for subsequent amplification of the genomic positive strand (+) RNA.
2. RNA transcription progressing via synthesis of subgenomic (sg) (-)RNA intermediates that are subsequently transcribed into mRNA. Some of the (-)sgRNA species are made through discontinuous processes that include template shifting. Once synthesized, the viral mRNAs are 5'-capped and 3'-polyadenylated to enable efficient translation of viral proteins.

All steps of the coronavirus RNA synthesis are carried out by the viral RTC encoded by approximately one third of the viral genome^{9,10}. The RTC is an assembly of at least nine viral nonstructural proteins (nsp) known as nsp7 through nsp16, as well as some less characterized host factors. Core catalytic function of the RNA synthesis is performed by RNA-dependent RNA polymerase (RdRp; nsp12) and two of its co-factors (nsp7 and 8). Coronaviruses also encode for RNA proofreading 3'-5' exonuclease function, carried out by nsp14, that increases the fidelity of replication and maintains genetic stability of the large coronavirus genome. The 5'-capping of RNA is performed by two methyl-transferases (nps14 and nsp16). Table 1 summarizes the viral RTC proteins and their functions.

Table 1. Virally encoded components of coronavirus RTC

Coronavirus protein	Function in RTC
Nsp7	RdRp cofactor
Nsp8	RdRp cofactor
Nsp9	RNA binding protein, capping regulator
Nsp10	Cofactor of Nsp14 and Nsp16
Nsp12	RNA-dependent RNA polymerase, capping
Nsp13	Zn-binding RNA helicase/RNA 5'-phosphatase
Nsp14	3'-5' exonuclease; N7-methyltransferase
Nsp15	Uridylate-specific endoribonuclease
Nsp16	2'-O-methyltransferase

Viral nucleic acid synthesis has been successfully targeted by a wide range of small molecule direct-acting antivirals, many of which have been developed into commercial drug products and are widely used in clinical practice¹¹. Examples include inhibitors of HIV reverse transcriptase (a DNA polymerase), hepatitis C virus (HCV), hepatitis B virus (HBV), and herpesvirus DNA polymerases. While some of these enzymes can be targeted effectively by non-nucleoside allosteric inhibitors (e.g., non-nucleoside reverse transcriptase inhibitors for HIV), nucleoside and nucleotide analogs mimicking the natural nucleic acid building blocks remain the most abundant class of viral replication inhibitors¹².

Potent and selective antiviral inhibitors of coronaviruses including SARS-CoV-2 have been identified among nucleoside and nucleotide analogs with known broad-spectrum antiviral activity, several of which quickly progressed into clinical testing for the treatment of COVID-19 (Figure 2). Table 2 lists a summary of profiles of these agents. Each candidate is a pro-drug that requires intracellular activation to a triphosphate form that is incorporated into viral RNA, resulting in effects including delayed RNA termination, RNA mutagenesis, and/or second RNA strand synthesis stalling.

VIRAL REPLICATION MACHINERY

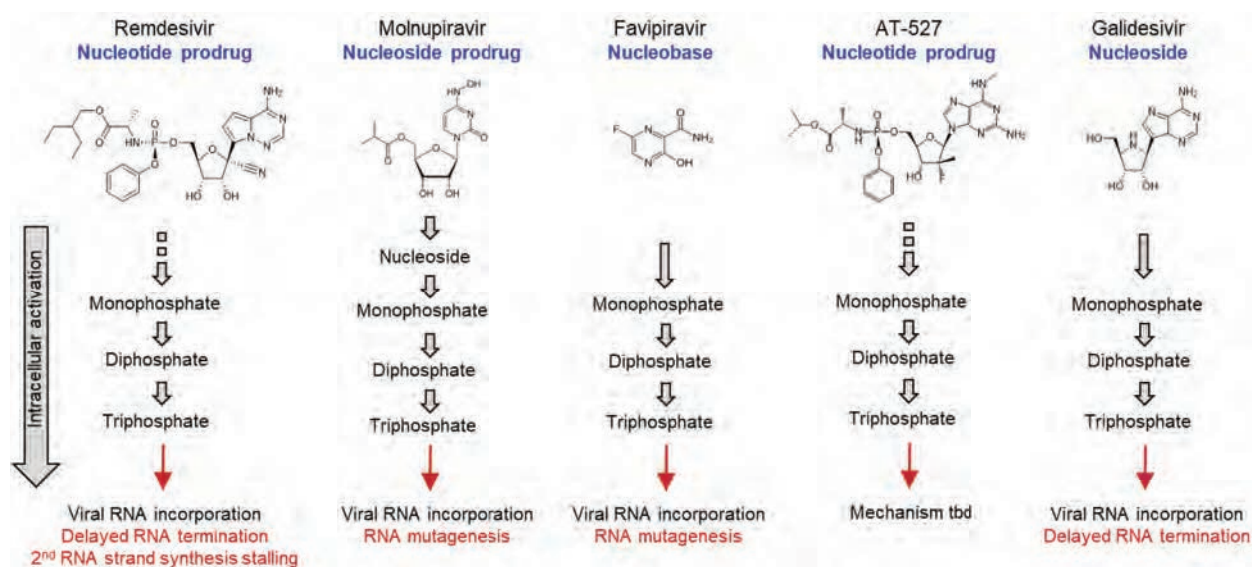


Figure 2. Structures of nucleoside analogs for the treatment of COVID-19, and their metabolic activation.

Table 2. Nucleoside analogs for the treatment of COVID-19. Status as of November 2020.

Nucleoside drug	Form	Mechanism of action	In vitro activity EC50 [μ M]	Animal model efficacy	Clinical dosing	Development status (Target population)
Remdesivir (GS-5734)	Nucleoside monophosphate prodrug	Delayed RNA chain termination	0.01 - 1.5	NHP, mouse	IV QD 200/100 mg	Approved in US & 50+ countries (Hospitalized); Phase 3 (Outpatient)
Molnupiravir (EIDD-2801; MK-4482)	Nucleoside prodrug	Viral RNA mutagenesis	0.05 - 0.3	Mouse	Oral BID 200-800 mg	Phase 2/3 (Outpatient and Hospitalized)
Favipiravir (T-705)	Nucleobase	Viral RNA mutagenesis	60 - 250	Hamster	Oral BID 1800/800 mg	Approved in Russia; under review in Japan
AT-527	Nucleoside monophosphate prodrug	tbd	0.5 (EC ₉₀)	tbd	Oral BID 550 mg	Phase 2 (Hospitalized)
Galidesivir (BCX-4430)	Nucleoside	Delayed RNA chain termination	58 - 68*	tbd	IV	Phase 1b

* In vitro activity against SARS- and MERS-CoVs. TBD = to be disclosed.

Remdesivir is a phosphoramidate prodrug that liberates an adenosine nucleotide analog within cells (the core nucleoside being GS-441524)¹³. Remdesivir was developed by Gilead Sciences with support from several U.S. Government organizations. Remdesivir was shown to be active against Ebola virus, and its safety profile was demonstrated in Ebola clinical trials. In several randomized clinical trials, remdesivir has demonstrated efficacy in hospitalized COVID-19 patients by reducing disease progression and accelerating time to recovery. In October 2020, remdesivir was approved by FDA as the first treatment for COVID-19^{14,15}.

Molnupiravir (EIDD-2801) is an orally bioavailable prodrug of N6-hydroxycytidine. It was developed at Emory (University) Institute for Drug Discovery (EIDD) as part of a program against Venezuelan equine encephalitis virus, and a Phase I trial was planned against influenza in 2019. As SARS-CoV-2 emerged, molnupiravir showed potent anti-SARS-CoV-2 activity both in vitro and in animal models¹⁶. It is currently in Phase 2-3 testing both in out-patient settings and in hospitalized COVID-19 patients.

Favipiravir, a guanine base analog, is a drug product approved in Japan (2014) for the treatment of influenza infections due to novel strains not responsive to other available agents¹⁷. It is also under regulatory review for the treatment of COVID-19 in Japan and has recently been approved for the same indication in Russia. It is active against SARS-CoV-2 in models, and is being tested in several advanced clinical studies around the world¹⁸. While it can be administered orally, it is substantially less potent than molnupiravir and remdesivir in vitro, which is reflected in a much higher dose required for favipiravir.

AT-527 is a prodrug of a guanosine nucleotide analog, developed by Atea Pharmaceuticals¹⁹. AT-527 was developed against the HCV RdRp, and safety was demonstrated in an HCV Phase 1-2 clinical trials in healthy and HCV-infected subjects. AT-527 was recently found to be active in vitro against SARS-CoV-2, and is currently being tested in Phase 2 in patients hospitalized with moderate COVID-19 disease²⁰.

Galidesivir is an adenine nucleoside analog developed by BioCryst for HCV, but it has broad-spectrum activity against RNA viruses and has been in development for treating deadly filoviruses (e.g., Ebola, Marburg)²¹. It has shown relatively weak activity in vitro against SARS- and MERS-CoVs and is slated for testing in a small proof-of-concept Phase 2 study in Brazil. It is the least characterized molecule among the SARS-CoV-2 nucleoside inhibitors.

It is worth noting that all the drug candidates described above existed before the COVID-19 pandemic, and novel chemical matter developed specifically for the SARS-CoV-2 replication

machinery has not yet been disclosed. While diverse in structures as well as formulations administered (see Figure 1 and Table 2), all SARS-CoV-2 nucleoside inhibitors require intracellular activation to their nucleoside triphosphate (NTP) metabolites, which then interact with the active site of coronavirus RdRp and are incorporated into viral RNA. The metabolic pathways leading to the respective NTP metabolites are cell-type dependent and differ for each molecule, as do their molecular mechanisms of viral RNA synthesis inhibition. Once incorporated into viral RNA, the metabolites of remdesivir and galidesivir cause delayed RNA chain termination, in the case of remdesivir template-dependent inhibition has also been shown, while those of molnupiravir and favipiravir act as specific viral RNA mutagens due to their capability of promiscuous base-pairing²². The mechanism of action for AT-527 is less well characterized and structural as well as biochemical data suggest that it might not interact very effectively with SARS-CoV-2 RdRp.

Challenges and Opportunities

Nucleoside and nucleotide analogs represent the most extensively explored class of antivirals with well understood advantages and limitations that both are related primarily to their function as mimetics of natural nucleotide molecules. Because the active sites of viral RNA polymerases to which the active NTP metabolites bind are often highly conserved within as well as across multiple viral families, many nucleosides exhibit broad-spectrum antiviral activity. Remdesivir, favipiravir, and molnupiravir are good examples of broad-spectrum antivirals with pan-coronavirus activity that may be leveraged as a part of future pandemic preparedness not only for the treatment of COVID-19, but likely also for other existing and newly emerging coronaviruses. The similarity of antiviral nucleoside analogs with natural substrates can also represent a significant liability, mainly because of their potential for off-target effects, such as impairment of mitochondrial functions through the inhibition of (host) mitochondrial RNA polymerase. Some nucleoside analogs might require prodrug strategies using established principles of medicinal chemistry to optimize oral delivery (bioavailability). In the case of SARS-CoV-2, the primary target tissue is the lung, and effective distribution into target cells irrespective of administration route is required.

Besides the inhibition of nsp12 (RdRp) by nucleoside analogs, the highly dynamic and multi-functional coronavirus RTC offers variety of other opportunities to interfere with viral RNA synthesis, including the other critical catalytic enzymatic functions present in RTC, such as the nsp13 helicase, nsp14 and nsp16 methyltransferases, or nsp14 exonuclease. In addition, the concerted function of this multiprotein complex relies on a multitude of specific protein-protein interactions, many of which have been mapped across the spectrum of coronaviruses including SARS-CoV-2. These efforts can be greatly facilitated by structural information generated by X-ray crystallography and cryo-electron microscopy studies conducted with many of the RTC proteins

and their complexes, including the nsp15 endonuclease, nsp14/10 and nsp16/10 methyltransferases, and particularly the multiprotein RdRp complex of nsp12/nsp7/nsp8 together with a dimer of nsp13 helicase. This wealth of structural information can facilitate the identification of critical interfaces and conserved pockets that might be amenable to targeting with small molecule inhibitors.

These alternative RTC targets offer the opportunity for non-nucleoside drug-like molecules, with the potential for candidates with desirable pharmacokinetic properties, like oral bioavailability, low protein binding, and adequate delivery to the respiratory tract. Unfortunately, the current state of the field underscores a complete absence of any validated small molecule inhibitors targeting some of these critical replicative functions, which in turn hinders advancement towards optimization of any new class of RTC inhibitors and their rapid progression into clinical testing. Therefore, the drug discovery process will rely on de novo high throughput screening approaches of suitable small molecule libraries (either pooled or arrayed). To that end, a number of recombinant RTC proteins have been cloned, expressed, and functionally characterized. Several options for functional biochemical assays exist depending on the desired format and preferred mode of read-out particularly for the catalytic complex of nsp12/nsp7/nsp8 RdRp. Similarly, functional assays have been established for coronavirus exonuclease as well as methyltransferases.

To effectively target some of the critical protein-protein interfaces, the field can employ a wide array of screening techniques for small molecule ligand binding, some of which are quite suitable for large compound libraries. Options include high-throughput affinity selection mass spectrometry (ASMS) DNA-encoded libraries (DELs), or surface plasmon resonance (SPR) assays. Alternatively, computational docking algorithms for putative binding sites can be employed for screening of vast virtual small molecule libraries. The major advantage of these approaches is speed and the number of potential candidate molecules identified, but these techniques need to be always coupled with relevant functional testing assays to triage the hits and validate their functional relevance. Newly-developed cell-based replicon assays for SARS-CoV-2 should be valuable to characterize activity of candidate compounds and certify cell-based activity and activation of candidate pro-drugs^{23,24}.

As will be discussed elsewhere, work remains to identify optimal target combinations with RdRp/RTC inhibitors (both small molecule antivirals and other modalities/approaches), and the patient populations most likely to benefit depending on individual drug product profiles, to maximize clinical effectiveness in the treatment and/or prevention of COVID-19.

SESSION 2

PROTEASES (VIRAL AND HOST)

Moderator:

Dr. Annaliesa Anderson, Pfizer

Panelists:

LTC Charlotte Lanteri, Walter Reed Army Institute of Research

Dr. Andrew Mesecar, Purdue University

Dr. Jennifer Nwankwo, 1910 Genetics

Dr. Celia Schiffer, University of Massachusetts Medical School

The SARS-CoV-2 Infection Cycle Requires Host and Viral Proteases

SARS-CoV-2 uses both host and viral proteases in its replication cycle (see Figure 1 in Overview). The generalizability of host proteases makes them a good target for many distinct coronaviruses that tend to use similar host factors. The spike proteins of SARS-CoV and SARS-CoV-2 have commonalities, such as sequence similarities and multi-basic proteolytic cleavage sites at both the S1 and S2 residue site as well as the S2 prime residue sites where host proteases (such as furin and TMPRSS2) cleave to facilitate viral entry. Host proteases do not mutate as easily as the viral proteases.

Host proteases TMPRSS2 and furin have a role in cleaving spike protein between S1 and S2 to reveal the fusion peptide and helical repeat domains that are necessary for viral entry^{25,26}. Currently two compounds are being investigated that potentially inhibit the host proteases and could be used as COVID-19 treatments: camostat and nafamostat. Both are repurposed drugs that are approved in Japan for the treatment of several conditions including pancreatitis²⁷. Since these compounds have existing human safety data, late-stage clinical studies for COVID-19 treatment are underway.

Drug discovery efforts to identify new host and viral proteases are actively being pursued. These include two efforts being led by the panelists in this session. The group at 1910 Genetics (founder Dr. Jen Nwankwo) is using machine learning design to screen millions of compounds to identify a suitable chemical substrate for host TMPRSS2 inhibitors. A second AI approach to identify viral protease inhibitors, led by LTC Lanteri at Walter Reed Army Institute of Research (WRAIR), combines X-ray crystallographic data with high performance machine learning.

Viral Protease Inhibitors (PIs) Have Been Used to Treat HIV and HCV Successfully

The first viral protease inhibitors were identified in the search for HIV cures²⁸. The HIV protease inhibitors are some of the first examples of structural based design, a move away from the traditional high throughput screening approaches that had been previously deployed for drug discovery efforts. A similar approach was also used for HCV and was so successful it was able to provide a cure for what was previously a chronic disease.

Structural design was first applied to coronaviruses in 2003 during the SARS epidemic caused by SARS-CoV-1 with the discovery of the first protease inhibitors specifically designed for coronaviruses. Both the public health measures that led to control of SARS-CoV-1 and the resulting lack of patients for clinical studies meant that that these drugs were never developed; it is not yet known if this approach will work for SARS-CoV-2, though there is promising in vivo data of these compounds against coronaviruses with similar proteases.

Two SARS-CoV-2 Cysteine Proteases can be Targeted for Potential COVID-19 Treatment

There are two SARS-CoV-2 encoded cysteine proteases that are potential antiviral targets: a papain-like protease and a 3C-like protease. These proteases are responsible for cleaving the polypeptide that is translated after the viral RNA enters the host cell. The single polypeptide makes up the machinery that the virus needs for replication. If it is not cleaved into the 16 individual non-structural proteins, the virus cannot replicate. The papain-like protease is a multifunctional enzyme that is also associated with pore formation and immune modulation²⁹. The 3C-like protease, which is also known as the main protease, functions as a dimer and has a single role that is to cleave the polypeptide at 11 sites³⁰.

HCV and HIV Drugs Have Limited Utility for COVID-19 Treatment, Current Potential Antiviral Inhibitor Candidates

Early in the pandemic, steps were taken to investigate whether existing HIV and HCV protease inhibitors could be redeployed to treat COVID-19 patients. There are considerable differences in the structure of the different proteases, so this approach led to little success, although some inhibition had been observed. Clinical studies are still in progress with the HIV drug ritonavir-boosted lopinavir, but an early controlled trial in COVID-19 patients in China failed to find evidence of antiviral effects or clinical benefits, and subsequent trials have confirmed the lack of therapeutic efficacy in hospitalized COVID-19 patients. Also, it is unclear what is its mechanism of action. Lopinavir and isotretinoin are examples of drugs repurposed for potential antiviral therapy. A Phase 1 study of PF-07304814 (termed PF-'814) is ongoing. This drug was specifically designed to inhibit coronavirus proteases. Several preclinical protease inhibitors are also under investigation and extensive screening for additional protease inhibitors is ongoing.

Example of 3-CL PI Development: Pivoting to a Pro-Drug Candidate PF-007304814 to Enable Clinical Dosing of Therapeutic Concentrations of Active Drug Candidate PF-0835231

In January 2020, as the potential force of the COVID-19 pandemic became apparent, Pfizer restarted its coronavirus PI development program that had been conducted in response to the SARS epidemic more than 15 years earlier. After comparing sequences of the two coronaviruses, a high degree of similarity was observed between the 3CL proteases, suggesting that Pfizer's lead compound PF-0835231 (termed PF-'231) could be developed and then potentially be deployed against SARS-CoV-2.

The following 8 months (to October 2020) saw the usual long development program dramatically compressed with the help of many coronavirus experts in the field to generate data for evidence that the candidate drug was highly potent and specific for the inhibition of coronavirus 3CL proteases including that from SARS-CoV-2. When it was recognized that the compound's potential pharmacokinetic exposure was suboptimal, teams of chemists quickly developed the phosphate

prodrug PF-'814³¹. This prodrug increased the exposure of the drug to allow for effective dosing in clinical studies, the first of which was initiated in September 2020 (NCT04535167).

PF-0835231 SARS-CoV-2 Protease Inhibitor has Additive Activity with Remdesivir In Vitro Due to Differentiated Mechanism of Action and Has the Potential to be Broadly Cross Reactive Across Coronaviruses

Remdesivir and Pfizer's PF-'814 have distinct antiviral mechanisms of action. Since antiviral treatment has benefitted from combination therapy, the antiviral activity of combinations was explored in vitro. The PF-'231 starting point showed potent single agent EC₉₀ in a SARS-CoV-2 antiviral assay, indicating potential clinical efficacy for viral protease inhibitors. Combining PF-'231 with remdesivir increased the observed in vitro potency. These in vitro data suggest that a combination may result in control over the virus with reduced amounts of each compound.

Collectively these in vitro data suggest Pfizer's prodrug has the potential to be effective as a single or a combination agent against SARS-CoV-2. In addition, it appears the compound has the potential to be a pan-coronavirus drug that can be deployed in the event of potential future coronaviruses that emerge as a result of additional species crossover events. The data generated suggests that this potential first-in-class protease inhibitor may provide the best opportunity to show meaningful antiviral activity to help treat COVID-19 patients. Two clinical studies are ongoing: one is a Phase 1 single ascending dose (SAD) study in healthy volunteers (NCT04627532), and a second is a Phase 1b in patients (NCT04535167). A pivotal Phase 2/3 study is planned to start in early 2021, with a projected approval in the second half of 2021.

PL^{pro} Has Other Functions in Addition to Protease Activity that PL^{pro} Inhibitors May Also Prevent

Coronavirus PL protease inhibitor development is not as advanced as the 3CL^{pro} inhibitors. Inhibitors targeted to this protease may provide a unique opportunity to not only inhibit viral replication via inhibition of cleaving the nsp3, but also inhibit nsp3 from antagonizing the NF-κB and IRF3 host innate pathways via deubiquitination and deISGylation as well as removing ISG15 from proteins in the Jak/Stat pathway. The dysregulation of these pathways may contribute to excessive cytokine release from infected cells. In addition, Nsp3 has recently been shown to be part of a molecular pore in double-membrane vesicles (DMVs). The DMVs form after viral infection as a result of nsp3, nsp4 and nsp6 embedding in the rough endoplasmic reticulum and reshaping it.

Challenges and Opportunities

The challenges and opportunities for the discovery and development of SARS-CoV-2 protease

inhibitor treatments were discussed by the panel, with a focus on how advanced screening methodologies are being used to accelerate screening and the importance of structural analysis to identify potential resistance challenges and facilitate compound discovery. Current research into protease inhibitors has been enhanced by consortia and collaborations. For example, work conducted by the Center for Structural Genomics enables the acceleration of drug design and validation programs.

With each new mechanistic insight come new targets, including host factors. These targets provide an opportunity for rational drug design accelerated by artificial intelligence (AI) that can be deployed at all stages of the early drug discovery process, from novel hit discovery to identification and optimization. For example, a 1910 Genetics AI platform allows the rapid screening of a billion-chemical library in less than 6 hours to identify hit compounds. Hit compounds are synthesized to ensure that they are not cytotoxic and then confirmed as actively inhibiting viral entry into Vero E6 cells engineered to overexpress TMPRSS2.

With the arrival of the COVID-19 pandemic, WRAIR scientists initiated a discovery program in small molecule therapeutics directed at SARS-CoV-2, with the goal of developing a small molecule therapeutic that can treat or prevent SARS-CoV-2 and other circulating coronaviruses in anticipation of the next emerging virus. LTC Charlotte Lanteri explained that this involved a significant pivot for the WRAIR Experimental Therapeutics (ET) Branch, the Department of Defense's (DoD's) sole drug development group, into a new therapeutic area of developing antivirals. The WRAIR ET team leveraged their inherent drug discovery and development capabilities and expertise for developing malaria and antibacterial drugs to address COVID-19.

WRAIR partnered with the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) scientists who established a high throughput in vitro SARS-CoV-2 Vero cell-based test system at their biosafety level-3 (BSL-3) labs. As part of this DoD COVID-19 Small Molecule Therapeutics effort, collaborators at the Southwest Research Institute in San Antonio, TX, in conjunction with the DoD's High Performance Computing Modernization Center, applied a proprietary AI/machine learning algorithm to screen >41 million compounds against two SARS-CoV-2 targets: the main protease and Spike protein Receptor Domain. The virtual screens were built based on published crystal and cryo-electron structures, as well as X-ray structures of the virus produced by the WRAIR Emerging Infectious Diseases Branch. The DoD group screened a library of >41 million compound structures in a matter of weeks. Compound structures came from a diversity of sources, some of them were existing drugs that may be repurposed for SARS CoV-2 and other coronaviruses. Others were novel chemical matter from a large repository of compounds that had already been analyzed for drug like properties. In contrast to traditional in silico

method introduces the ligand to the target with no pre-conceived notions of binding pose. Their ultimate goal is to develop a pan-coronavirus drug; as such, virtual screen hits were also screened against an analogous target (RBD) from SARS-CoV-1 and MERS to select compounds with the greatest overlap in predicted binding. From this large scale initial screen, they rapidly down-selected approximately 800 compounds within a few weeks, dramatically shortening the traditional years-long discovery phase. To date, testing of in silico hits resulted in a 9% hit rate in USAMRIID's SARS2 antiviral Vero cell screen. This outcome is orders of magnitude better than more traditional high throughput put methods. The combination of AI and then high-throughput screening using an invitro assay has been key for accelerating the discovery of potential new coronavirus treatments. The molecules are currently being studied from a medicinal chemistry perspective including determination of their metabolic properties, safety evaluation and pharmacokinetics in mouse models.

PL^{pro} Has Other Functions in Addition to Protease Activity that PL^{pro} Inhibitors May Also Prevent

Dr. Mesecar of Purdue University described working on PL^{pro} and M^{pro} after the SARS-CoV-1 pandemic in 2003-2004 with a collaborative team³²⁻³⁵. At that time, the papain-like protease was an overlooked protease with no established structure. Cleavage of ubiquitin off host cell proteins was also identified as another potential role for the PL^{pro}. Dr. Mesecar and his group confirmed that PL^{pro} was capable of utilizing ubiquitin substrates. He evaluated interferon-stimulated gene 15 (ISG15) and determined it is also capable of hydrolyzing 7-amino-4-methylcoumarin (AMC) of ISG15 causing its removal from ubiquitin in host cells. Understanding the complete role of PL^{pro} antagonism of an aspect of host innate immune response will be important to the successful use of PIs of this protease.

Dr. Mesecar and colleagues elucidated the structure of PL^{pro} and found that it was indeed a ubiquitin-specific protease (USP). They then conducted a high-throughput screen of 50,000 compounds, identifying two potential compound templates for anti-viral drug development. These non-covalent inhibitors had the ability to target only a certain subclass of coronaviral papain-like proteases selectively, making it possible to target papain-like proteases selectively. To date these compounds have shown efficacy in a non-SARS-CoV-2 coronavirus animal model.

Design of protease inhibitors to pre-emptively avoid Drug Resistance:

Viral proteases are key drug targets and have been one of the most successful structure-based drug designs. However, drug resistance can come from mutations in the active site, or remote changes that alter the flexibility of the protease³⁶⁻³⁸. The necessity of the protease to cut a series of cleavage sites is a key evolutionary constraint. Dr. Celia Schiffer's laboratory at University of

PROTEASES (VIRAL AND HOST)

Massachusetts Medical School, for example, have focused on this evolutionary constraint of substrate recognition and processing and how to combine that evolutionary constraint to preemptively avoid drug resistance. Inhibitors that fit within the substrate envelope are less likely to produce resistant strains because the mutation impacting these inhibitors will simultaneously impact the recognition and processing of the majority of the substrates. Dr Schiffer's team has previously demonstrated this approach with HIV and HCV proteases^{39,40}. Similar to other viruses, SARS-CoV-2 has already evolved during the current pandemic. As the SARS-CoV-2 viral proteases both recognize multiple substrates, the substrate envelope approach is being pursued as a way to pre-emptively avoid resistance. Combining protein crystallography, medicinal chemistry, and computational methods to SARS-CoV-2 3CL^{pro} and PL^{pro} with a collaborative team at University of Massachusetts Medical School, the aim is not only to have inhibitors that retain effectiveness during the current pandemic but are potent against any future coronaviral outbreaks.

SESSION 3

EMERGING TARGETS, EMERGING MODALITIES

Moderator:

Dr. Kara Carter, Evotec*

**Current affiliation: Dewpoint Therapeutics*

Panelists:

Dr. David Baker, University of Washington

Dr. Lillian Chiang, Evrys Bio, LLC

Dr. Matthew Disney, Scripps Research Institute

Dr. Kumar Saikatendu, Takeda Pharmaceuticals

Dr. Marla Weetall, PTC Therapeutics, Inc

As discussed above, small molecule inhibitors of essential viral enzymes, such as polymerases and proteases, are well validated approaches for antivirals. However, as with all complex viral diseases, multiple therapeutics to address different aspects of the SARS-CoV-2 viral life cycle and in different COVID-19 patient populations are needed to have the ability to effectively treat patients. Several new modalities have arisen for therapeutics in general over the last several years including RNAi, CRISPR/Cas9 and other targeted nucleases, bifunctional molecules, and others. Dr. Matt Disney of Scripps Research Institute described how sections of viral genomes in general, and SARS-CoV-2 in particular, form constrained secondary structures that are specifically targetable by small molecules. Such regions of the SARS-CoV-2 genome include the 5' untranslated region and the frame shift motif. Small molecule screens have been conducted and identified binders to these elements⁴¹. These binders have been used as the basis of a chimeric compound that can target a host ribonuclease to the viral genome and ultimately lead to its degradation. Such a mechanism has demonstrated potent antiviral activity. In addition, Dr. David Baker of the University of Washington described his work generating mini-proteins that specifically bind to the receptor binding domain of the viral spike protein with potent nanomolar antiviral activity⁴². These mini-proteins were computationally designed and are able to be produced in large quantities that could lead to minimal cost of goods. In vivo, these mini-proteins delivered either intranasally to hamsters or as Fc-fusions by injection to mice, had protective effects when the animals were challenged with virus.

Both of these newer modalities have potential significant advantages. First, drug discovery can begin as soon as a new genome is sequenced (a parallel with vaccine development). For mini-proteins, computational methods lead to the synthesis of small numbers of molecules for testing. For RNA binders, analysis and comparison to sequences of related viruses can accurately predict genome sequences/structures to start screening for small molecule binders. The mini-proteins, as mentioned above, can be developed with different formulations for different routes of administration and have good potential for low cost. Additionally, at least in the case of the mini-protein generated against SARS-CoV-2 spike, the molecule is thermostable and can be stored at room temperature. The RNA targeting small molecules may have a lower incidence of mutation given there are only three options of nucleotides to be incorporated into the RNA for such mutations, and to maintain functional structure of the RNA, even substitution by those three options may not be tolerated by the virus. Both of these modalities, though, lead to molecules that will likely have activity limited to viruses with close sequence homology in the viral target.

Complementing the advances in new modalities is the identification of novel targets for antivirals beyond the traditional viral enzymes such as polymerases and proteases. Both systems biology studies^{43,44} and compound repurposing screens^{32,45-49} have identified a number of potential

host cell targets to be explored for validation and ultimately drug discovery and development. One example is dihydroorotate dehydrogenase (DHODH), an essential host enzyme in de novo pyrimidine biosynthesis. Studies to identify pathways and/or targets can vary in their methodology, including use of different cell types and tagging or knock-out of proteins, which can lead to conflicting results. The public visibility and combination of large data sets may allow identification of potential targets. These targets become actionable as they are verified with the use of probe molecules to test the therapeutic hypothesis, and demonstration of activity in multiple orthogonal assay systems.

Drug discovery focused on host cell targets is anticipated to present multiple opportunities that will require verification. As opposed to direct acting antivirals that may have a less risky developmental path, drugging host proteins or processes presents less potential for resistance emergence and should be effective irrespective of the viral load. Additionally, such molecules tend to be more refractory to drug block release, meaning that after removal of the drug, the changes that the drug induced in the host cell are maintained by extending the antiviral effect. Particularly compelling cellular targets are those engaged in stress activated signal transduction and the dysregulated metabolism induced upon viral infection^{50,51}. For example, Evrys Bio, LLC is developing inhibitors of host SIRT2, which have demonstrated potent and durable antiviral activity across a number of viruses^{52,53}.

As host genes are nominated as druggable candidates, rigorous validation of the target and modality, translating active molecules to drugs and ultimately to medicines requires focus on physical properties, pharmacokinetics, formulation, and other key pillars of drug development. These new modalities and targets do not replace small molecule drug discovery against traditional, validated targets like polymerases and proteases. Rather, they are expected to complement those efforts and increase the diversity of agents that can be used to treat COVID-19 patients. In some cases, these approaches may provide more rapid discovery and development of drugs in a pandemic setting. Additionally, the breadth of activity and potency, even in the face of high viral load, of drugs targeting cellular proteins can complement more traditional antivirals. These new modalities and targets should certainly be explored as part of the SARS-CoV-2 response.

SESSION 4

PRECLINICAL TOOLS

Moderator:

Dr. Pei-Yong Shi, University of Texas Medical Branch

Panelists:

Dr. Sara Cherry, University of Pennsylvania

Dr. Emmie de Wit, NIAID/Rocky Mountain Laboratories, NIH

Dr. Jules O'Rear, US Food and Drug Administration

Dr. Timothy Sheahan, University of North Carolina

Dr. Hugh Smyth, University of Texas at Austin

Biologically and clinically relevant models are required for the evaluation of antiviral drug candidates in order to obtain the most meaningful and informative preclinical data. Both in vitro (cell-based) and in vivo (animal) models of SARS-CoV-2 infection have been developed to evaluate antiviral activity and therapeutic efficacy of candidate drugs. Various in vitro systems have been used to identify potential COVID-19 therapeutics in the screening of drug libraries comprised of approved drugs with the hope of repurposing in addition to the screening of compound libraries to identify new antiviral compounds to then be optimized into a more drug-like form through medicinal chemistry. Animal models of SARS-CoV-2 infection that recapitulate viral tropism and COVID-19 disease pathology are required to ensure that pre-clinical candidates are active at the target site (e.g. lung) and to assess antiviral combinations. As SARS-CoV-2 is a respiratory virus, formulation of oral or intranasal delivery of therapeutics will be most impactful as dosing would not require assistance from a medical professional. Preclinical animal models are essential to determine if therapeutic candidates have antiviral activity, optimal pharmacokinetic profiles, and mitigate SARS-CoV-2 pathogenesis.

In vitro Models

For cell-based antiviral testing, both wild-type virus and those engineered to express reporter genes like luciferase or fluorescent proteins have been used to screen compounds for antiviral activity⁵⁴. Antiviral activity and potency have been determined by multiple techniques including through the measurement of reporter gene products, direct measure of infectious virus by classical virological techniques, measurement of viral cytopathic effect in cell monolayers, and immunostaining-based measurement of viral proteins via high-content imaging which can also monitor cytotoxicity⁴⁵. To most efficiently triage compounds, early removal of toxic compounds is preferable. Compared with wild-type virus, reporter virus assays typically have a higher dynamic range and potentially higher throughput capacity. High-throughput assay formats also allow the screening and assessment of antiviral combinations in cell culture, which require a large number of experimental conditions to be evaluated⁵⁵.

The target cell type employed is also critical to accurately predict antiviral activity of candidates in humans. A number of seemingly simple considerations, including the species of origin of a given cell line (e.g., human versus other animal-derived cell lines), and the tissue of origin (e.g., respiratory epithelial versus kidney or colon) significantly impact the relevance of antiviral candidate potency. For example, the FDA-approved RdRp inhibitor remdesivir IC₅₀ values differed by 1,000-fold when remdesivir was tested in Vero cells (African green monkey kidney epithelial cell line, weak activity) compared with primary human airway culture (potent activity)⁵⁶. This is likely due to differences in cellular enzymes that transport and/or metabolize the prodrug into its pharmacologically active triphosphate form. Conversely, chloroquine and its derivatives

showed anti-SARS-CoV-2 activity in Vero cells, but not on primary human airway cultures or animals⁵⁷. These results underline the importance of selecting the right cell types for antiviral testing, and primary or multicellular airway models should be considered to confirm antiviral activity.

Reverse Genetic Systems

Reverse genetic systems have been developed for SARS-CoV-2, leading to the generation of both fluorescent and luminescent reporter viruses that can increase the throughput of screening assays with authentic virus particles at BSL-3^{58,59}. In addition, reverse genetics enables the phenotypic confirmation of drug resistance mutations derived through passage in cell culture or those that naturally arise in humans and drug target deconvolution in mechanism of action studies with newly discovered drug candidates. Functional genomics screening approaches such as CRISPRi can enable the identification of human (host) genes that play a cooperative role in mediating the viral replication cycle and may be druggable.

In vivo Models

Several animal models have been developed for SARS-CoV-2, including mouse, hamster, ferret, and non-human primates. A detailed list of SARS-CoV-2 animal models is maintained by the NIH ACTIV Preclinical Working Group at the NCATS OpenData Portal^{60,61}. For both small animal and non-human primate models, information on background, primary references, and viral model endpoints are provided.

The establishment of any animal model relies on that species being susceptible to infection by SARS-CoV-2⁶², and a secondary consideration is the species being amenable to maintenance in a vivarium setting. Although mice are typically used widely to study viral pathogenesis and antiviral efficacy due to their size, ease of use, available genetic models, and experimental tools, due to differences in the murine ortholog of the human receptor, angiotensin converting enzyme 2 (ACE2), SARS-CoV-2 spike cannot bind mouse ACE2 and cannot infect standard laboratory mice. To circumvent this issue, approaches have been taken to enable SARS-CoV-2 infection in mice. First, transgenic mice have been made that express human ACE2⁶⁴. Second, viral vectors have been used to deliver and overexpress human ACE2 in the mouse lung⁶³. Lastly, SARS-CoV-2 has been genetically adapted to utilize mouse ACE2 for entry resulting in a virus with high titer replication in the lungs of standard laboratory mice (i.e. BALB/c), loss of pulmonary function, severe end stage lung disease like ARDS and death⁶⁴. In general, any of these mouse models can be useful for antiviral testing. In addition, because the mouse-adapted infection model recapitulates multiple aspects of the human disease, this model may be more useful to study viral pathogenesis and genetically dissect host immune responses driving disease.

Hamster models have been developed to study viral replication, pathogenesis, and transmission^{65,66}. Unlike mice, wild-type SARS-CoV-2 robustly infects hamster respiratory tract, causing weight loss and lung pathology. The hamster model also recapitulates age-dependent disease severity. The kinetics of viral replication in young hamsters is faster than that in aged hamsters, whereas the immune response in aged animals lasts longer, leading to somewhat more severe disease. Moreover, hamsters can transmit the virus allowing for the study of SARS-CoV-2 transmission. Ferret models have also been established to study viral transmission. Compared with other animal models, the viral replication level and disease severity are milder in ferrets.

Several non-human primate models of SARS-CoV-2 have been reported, including rhesus macaque, cynomolgus macaque, and African green monkey⁶⁷⁻⁶⁹. In the rhesus macaque model, the infected animals develop mild to moderate clinical signs of disease, develop pulmonary infiltrates on radiographs, and virus shedding in nose and throat swabs is similar to that observed in COVID-19 patients. Although no non-human primate models have been established that recapitulate severe COVID-19, these models have been used successfully to show the efficacy of several direct-acting antivirals and antibody treatments.

In summary, antiviral models have been developed to support in vitro and in vivo drug discovery. However, limitations remain, in large part due to the biosafety considerations, to enable more efficient therapeutic development.

Challenges and opportunities

Data reproducibility and assay comparisons

One challenge to SARS-CoV-2 drug discovery and development is that different research teams have reported different activity, or lack of activity, for the same compound. One “side effect” of so many research groups converging on SARS-CoV-2 research has been the use of differing models and assays (described above). The adoption of common reference assays and criteria would set standards for the field. This would include recommendations on appropriate cell lines and reagents and making sure these are readily available to qualified investigators. For example, HIV repositories exist for small molecules, viruses and cell lines promoting standardization which has been beneficial to drug development (though this was implemented over a longer timeline, see for a general example the Biodefense and Emerging Infections Research Resources Repository, BEI Resources). Importantly, the reliance on a single assay model for demonstrating activity is not adequate, and orthogonal assays are essential. In addition, testing against standardized positive and negative controls in assays is also important.

Combination antiviral therapy

Combination antiviral therapy is expected to be critical to effectively treating infections and minimizing the development of resistant strains. Screening approaches can be used to identify potent synergism of antiviral candidates, and functional/genetic approaches can be used to predict drugged target combinations that should be effective. Moreover, any new therapeutic, especially other RdRp inhibitors, must be tested for antagonism against approved therapies, such as remdesivir, to ensure that unwanted impedance of antiviral activity does not occur in combination. Also initial clinical studies of potential drug-drug interactions with respect to human pharmacokinetics and tolerability are necessary.

In vivo models

Before the SARS-CoV and MERS-CoV outbreaks, very few models of coronavirus pathogenesis existed, but researchers responding to the SARS-CoV-2 pandemic rapidly took advantage of previous approaches and models developed for other emerging coronaviruses (e.g., human ACE2 transgenic mice). However, the numbers of researchers able to engage in research with an authentic emerging coronavirus is limited by the need to perform this work in a BSL-3 laboratory of which there are only a handful in the United States. Another major gap is that it remains difficult to study long-term effects of either treatment or disease in animal models. From an antiviral candidate perspective, “direct-acting” compounds against viral proteins can be applied to many animal models, but host-target antiviral candidates (for example, host proteases described earlier) rely on an understanding of the species homolog of the human gene. Current animal models are not adequate for studying extrapulmonary manifestations of disease. Studying comorbidities is also difficult in animal models, which is a major weakness given the pathology of COVID-19 in humans, and the anticipation that newly developed antivirals will be effective in reducing mortality in patients with such complicating comorbidities.

To date, the FDA has not been requiring animal model data for some clinical trials using agents with known activity during viral infections, and well-defined safety based on prior in-human use. However, antiviral activity information remains essential in instances when other data, on topics such as the mechanism of action or toxicity, are lacking. Mechanism of action and resistance data is of vital importance. In this context, the designation of a standard accepted in vivo model with gold standard positive and negative controls for assessing and comparing antiviral activity of a new therapeutic has not been designated.

SESSION 5

LESSONS FROM OTHER VIRUSES AND PREPARATION FOR THE FUTURE

Moderator:

Dr. Daria Hazuda, Merck & Co.

Panelists:

Dr. Jay Bradner, Novartis

Dr. Courtney Fletcher, University of Nebraska Medical Center

Dr. Frederick Hayden, University of Virginia

Dr. Hilary Marston, NIAID

There is a long history of antiviral drug discovery and development dating back to the 1960s. While these earliest efforts were largely empiric, the tremendous effort against HIV in the 1980s and 1990s was pivotal in the shifting emphasis to more target-based drug discovery and some of the earliest successes in structure-based and rational drug design. The success of antiretroviral therapy for HIV illustrated the critical importance of combination therapy and adherence in treating chronic infections and highlighted the value of fixed dose combinations, convenience, and tolerability. These fundamental lessons were subsequently relearned in the discovery and development of direct acting antiviral drugs for HCV.

HCV drug discovery also emphasized a target-based approach for the development of protease and polymerase inhibitors, but in the end, empiricism was also critical to this amazing success story. The HCV NS5a inhibitors, which became pivotal to most successful combination therapies, were discovered from agnostic cell-based screens that were only made possible by the development of replicon assays. The development of replicons for multiple diverse HCV genotypes was fundamental to achieving the breadth of activity needed to address both spectrum and resistance, also illustrating the critical value of having the right in vitro models to drive the drug discovery process.

Throughout the 1980s, 1990s, and 2000s, advances were also made in therapeutics for HBV, influenza, and various herpesviruses. Today there are more than 90 approved antiviral agents to treat a variety of both acute and chronic viral infections. While there are successful antiviral drugs across multiple distinct target classes, the largest number and broadest class of approved antiviral drugs are those which target viral polymerases. Polymerase inhibitors have been approved to treat infections across both RNA and DNA viruses as well as retroviruses and comprise a myriad of mechanisms, including substrate mimetics such as nucleoside and pyrophosphate analogs but also non-nucleoside or allosteric inhibitors. It is worth noting even nucleosides can exhibit distinct mechanisms of action, including chain termination, inhibition of translocation, and error catastrophe with unique implications for pharmacology and resistance.

In general, enzyme targets dominate the list of approved antiviral agents, but after polymerase, other enzyme targets are generally unique to different virus families making it challenging to find drugs that work broadly. The challenge of finding approaches that are effective across a virus or virus family is particularly true for viral entry targets, which can often vary even for a specific virus in cases where multiple receptors or modes of entry are observed. The best clinical illustration of this challenge is the development of CCR5 inhibitors for HIV, where having a diagnostic to differentiate CCR5 vs CXCR4 tropism was critical and became an impediment for the use of such

agents in the real world. CCR5 inhibitors are also one of the only examples of a successful antiviral development against a host target, another challenge for antiviral development generally.

Despite a tremendous track record of success, there are lessons from notable disappointments in antiviral drug development. There is always the potential for failure due to toxicity or pharmacology that plague drug development more generally, but in the case of antivirals, in some cases poor efficacy can be attributed to a drug's inability to address either inherent diversity or easily acquired resistance. One such example is the first generation HCV protease inhibitors that were obsolete by the time they launched, being replaced by agents with improved resistance profiles and spectrum. While in many cases, learnings from the initial clinical disappointments resulted in improved agents, an earlier understanding of diversity and resistance could have avoided many of these early mistakes. Therefore, understanding the impact of genetic diversity and resistance is important even at the earliest stages of target and lead selection.

While it is certainly preferable to develop agents which have a high barrier to the development of resistance, as seen in HIV and HCV, even agents with a low barrier to resistance development can work when combined if there is adequate pharmacologic coverage. The importance of combinations appears to be more important in treating chronic infections, where there is ongoing replication and a larger more diverse population as compared to prevention where dual and even monotherapy is highly effective. But even in chronic infection, monotherapy can sometimes work, as is the case in HBV, likely a consequence of both the biology of the virus and the specific agents.

It is important to note that resistance and antiviral efficacy are intrinsically linked to pharmacology. The following characteristics have been found to decrease the probability of the development of resistance: high bioavailability, high plasma and/or organ/tissue/site-of-action distribution, long elimination half-life, low intra- and interpatient variability, low probability as a victim or perpetrator of drug-drug interactions, and convenient dosing regimens (which promote high adherence and are forgivable of missed doses). To maximize the potential for broad impact and global use, oral agents with low cost of goods that are easy to formulate and also suitable for pediatric formulations are critical. For chronic therapy and prevention, drugs which are amenable to long-acting formulations are highly desirable. In addition, understanding where the agent needs to be delivered to treat or prevent infection is key. In respiratory infection for example, intranasal administration may not be sufficient to treat or prevent lower respiratory tract disease but may be useful for prophylaxis.

For both acute and chronic infections, timing is key. As has been shown in HIV, early treatment and prophylaxis lead to profound patient, public health, and economic benefit. The same has been

observed in the setting of preemptive therapy or post exposure prophylaxis. How soon post-exposure prophylaxis needs to be administered may vary between different viral infections and antiviral mode of action, but in all preclinical models and clinical studies of respiratory viral infections, sooner is better. In a pandemic setting, this is especially critical. For influenza, insufficient and delayed use of neuraminidase inhibitor treatment resulted in these drugs having a diminished impact on patient mortality during the 2009 pandemic despite their known effectiveness. The exceptions included countries like Japan that had high levels of antiviral coverage, including children and pregnant women.

A number of potential SARS-CoV-2 inhibitors are being considered for topical administration (e.g. intranasal, inhaled) to the respiratory tract. One issue for topically delivered respiratory virus antivirals is whether intranasal administration is sufficient, which in turn, depends on the principal site(s) of initial acquisition. Intranasal interferons (IFNs) protect against rhinovirus and likely common respiratory coronavirus illness. However, neither intranasal IFN nor intranasal zanamivir prevent natural influenza illness. In contrast, inhaled zanamivir is highly effective for prevention of influenza illness (75-80% in household contacts). It remains to be determined whether antivirals administered intranasally (i.e. protecting only the nose) might prevent some SARS-CoV-2 infections. Inhaled investigational agents like IFN-beta, which has shown some efficacy in hospitalized COVID-19 patients, and when available, inhaled remdesivir may prove effective in prevention and early treatment.

It is well established that antiviral drugs are highly effective in both treating and preventing infections, if they have the right pharmacologic properties, can be easily accessed, and administered to at risk populations. Having the appropriate in vitro tools and in vivo models to develop antivirals are key. Investing in the basic biology to develop these tools, assays, and models, understand the implications of resistance and overcoming the barriers which impede rapid access to the use of such drugs once developed, are critical for limiting future pandemics and outbreaks and requires a collaborative effort which crosses public, private, and governmental boundaries.

Preclinical Pharmacokinetics and Formulation

As with any preclinical drug development program, it is essential to understand the physicochemical properties of the drug compound and the resulting pharmacokinetic profile in order to improve chances of success in demonstrating in vivo efficacy. For example, Remdesivir is given intravenously due to poor oral bioavailability. Moreover, remdesivir required solubility enhancement using cyclodextrins due to its limited solubility⁷⁰ Preclinical formulation development should take into account drug solubility, stability, and absorption. In the case of

alternative routes of administration may also be considered. Notably, intranasal and pulmonary administration appears a promising strategy for SARS-CoV-2 antiviral delivery where ACE2 receptor distribution is implicated in initial infection and subsequent pathology⁷¹.

SUMMARY OF DISCUSSIONS AND PERSPECTIVES ON THE CHALLENGES AHEAD

Speakers:

Dr. George Painter, Emory University

Dr. Richard Whitley, University of Alabama at Birmingham

Overview of the Virus and Therapeutic Approaches

An overarching goal for the coronavirus antiviral program is the discovery of broadly active compounds that act at multiple sites in the virus replication cycle, and that target diverse coronaviruses (CoV) both in vitro and in animal models. The ability to administer drug by multiple routes (IV, oral, inhalation, and nasal) is highly desirable. A toxicity profile that supports prophylactic as well as therapeutic use is key. As the current pandemic emerged, the problems associated with clinical development in the absence of a clear understanding of the virologic and immunologic course of disease became apparent. Important considerations moving forward include determining acute viral burden, establishing the effective therapeutic window, and identifying potent combination therapies designed to improve efficacy and prevent resistance. Even with the availability of effective vaccines, antiviral drugs will be required to protect highly vulnerable populations with diminished immune function or who cannot take vaccines due to allergic reactions.

Targeting Viral Replication Machinery of SARS-CoV-2

Therapeutics against SARS-CoV-2 consist of many subtypes: antibodies, early inhibitors of entry, fusion inhibitors, RNA polymerase inhibitors, protease inhibitors, and virus release inhibitors, among others. These can be either host or viral targeted therapeutics. One of the most successful methods to mitigate SARS-CoV-2 replication is by direct acting antivirals; currently, those that target the replication machinery are of high interest. To date, progress has been made in the development of nucleoside analogs, as these are the only class of antivirals that have been approved clinically for COVID-19 (e.g., remdesivir)¹⁴. There are pros and cons of developing nucleosides. Those nucleosides in which the sugar is ribose, called “ribonucleosides,” have had the advantage in that they had been jump started from other viral indications. Advantages include: 1. they mimic natural substances; 2. in vitro work has suggested a high threshold of viral resistance; 3. the development pathway is well understood; and 4. tests are in place to detect off target toxicities. The fact that ribonucleosides tend to be broadly active make this family of therapeutics desirable for interrogation (both for now, and off-the-shelf for future pandemics)⁷²⁻⁷⁴.

It has proven difficult to establish structure-activity relationships for nucleoside/nucleotide analogs, consequently nucleoside discovery generally is a trial and error process. The current approach is to simply introduce modification on the base or ribosugar, and then test each derivative for activity. In addition, nucleosides require multistep metabolic activation for conversion to the 5'-triphosphate derivative in order to be inhibitory. This effort can be a challenge as metabolic activation varies between different cell types, which makes selection of assay types and cell types a challenge for downstream evaluation. Further, the delivery of nucleosides to the target tissue of interest can be a challenge. However, there is a wealth of historical data that

informs the selection of those nucleosides that provide a starting place for synthetic efforts. Nevertheless, chemistry can be challenging working through the many variations of modifications that are possible on base versus ribosugar. Nucleosides, because they mimic natural factors, also can act through different mechanisms, targeting aspects of the replication machinery of either host or virus (selectivity). While tools have improved the design of antiviral nucleosides, these compounds must be tested in whole cell screens and must be evaluated for the potential off-target effects (mitochondrial or host cell compartment interactions). Lastly, the design of alternative delivery options, e.g. such as oral delivery - can be challenging. Tactics to make all the information surrounding the work in nucleoside therapeutics more widely known across disciplines including the assays to determine antiviral effect must be made more generally available.

Proteases (Viral and Hosts)

An obvious target is viral and host proteases, as these proteins are important for viral entry and processing. Of benefit is that libraries of HIV and HCV protease inhibitors provide a logical starting point for synthesis. Repurposing protease inhibitors provides an invaluable and rich resource especially for combination therapies. Proteases have been demonstrated to be broadly druggable, and new pre-clinical candidates will emerge. Currently, success has been extremely limited in contrast to that which has been achieved with HIV and HCV infections

Emerging Targets and Emerging Modalities

Nucleosides are of interest because they function as directly interacting with replication processes within either the virus or host. However, there are numerous proteins that are part of the replication machinery, providing opportunities for other target strategies. Efforts to selectively target downstream proteins are now becoming well advanced. In addition, through the application of AI computational oversight to identify properties of likely drug candidates would be expected to be a major step forward, including helicase, endoribonuclease, exonucleases, and methyltransferase.

Knowledge of the lifecycle of SARS-CoV-2 virus indicates that there are numerous other targets of the viral or host genome or proteome that are largely unexplored and could be suitable for drug discovery. The fact that these targets are unexplored makes this route of therapeutic discovery and development more contingent on unknown factors. These unknown risks and benefits must be recognized, but it should not be a factor that impedes progress.

Preclinical Tools

When choosing the preclinical tools to test compounds in vitro and in vivo, the choice of cell type is crucial for cell assays. Orthogonal assays as well as AI are necessary tools to complement discovery efforts⁷⁵. In vivo models can be problematic but are ultimately helpful for therapeutic development. In vivo models can help determine if a drug is capable of engaging a target tissue. In particular, animal models provide a critical true tool for the assessment of drug distribution, e.g. site of viral replication is essential. Further, the half-life, oral bioavailability, metabolism, distribution, microsomal stability, etc. can all be evaluated in animal systems⁷⁶. However, animal models may fail to determine if metabolic differences can drastically alter the way a drug is metabolized. Further, it should be emphasized that a drop in viral burden is not a validated endpoint for efficacy. Each animal model has its own set of peculiarities and other models could be considered. Especially for SARS-CoV-2 (and other respiratory viruses), the air-liquid interface human airway cell model may provide insight into tissue site needs better than an animal model⁷⁷.

Clinical Trials and Lessons from Other Viruses and Preparation for the Future

When considering lessons from previous antiviral drug development, clinical trial design and execution should be carefully reviewed. Decrease in viral burden is not a traditionally validated endpoint with the exception of HIV, HCV, and cytomegalovirus (CMV) after hematopoietic stem cell transplantation (HSCT). However, for SARS-CoV-2 antiviral candidates, the lack of antiviral efficacy in early clinical studies of prevention or treatment of mild illnesses should raise concerns about advancing such candidates further. For agents demonstrating antiviral efficacy, what should antiviral clinical trial endpoints be? For SARS-CoV-2 the answer is dependent on the indication (e.g., pre-exposure or post-exposure prophylaxis, treatment) and target population (e.g., early treatment in outpatients to ameliorate illness, prevent disease progression, and/or reduce transmission; treatment in seriously or critically ill hospitalized patients). The logistical issues are many, such as defining diverse patient populations or, often forgotten, the staffing and supply needs to accommodate the appropriate trial. Standardization of platforms for each stage of development, including the clinical trials, would be a helpful step for all. This has been achieved in the pandemic response in part through creation of large pragmatic, adaptive trials in hospitalized COVID-19 patients like RECOVERY in the UK, WHO's Solidarity Trial, and ACTT and ACTIV in the US. These platforms have provided key outcomes data on therapeutics that helped (i.e., dexamethasone, baricitinib), those that did not (hydroxychloroquine, lopinavir-ritonavir, azithromycin) and those with inconsistent results (e.g., remdesivir). Availability of databases and algorithms for AI to provide computational oversight for in vitro and in vivo models and clinical trial design as well as openly sharing negative data among all research groups is useful to avoid rabbit holes that are a waste of time and money. Standardized endpoints would also enable "basket antiviral trials" that test multiple antivirals (or combinations) with a single placebo arm.

Ultimately, for the development of any drug, the most important factor is to guarantee that the lead is safe for human administration⁷⁸. As emphasized, the early stages of the drug discovery and development process should include considerations regarding what the final product insert will recommend as the indication. Considerations would include: will a compound be used for prophylaxis? What formulation options are required according to the route of delivery? Drug delivery to the site of viral replication/disease must be included in the development pathway and crucial treatment paradigm. Establishing these goals at the outset ensures the appropriate design, particularly outpatient or inpatient, with or without multiorgan involvement. In the medicinal chemistry field these are referred to as “target product profiles.” The breadth of knowledge critical for drug discovery and development is vast. A translational science approach with a combination of skill sets from virologic and immunologic backgrounds as well as chemistry, toxicology, and other fields are important factors of success.

Forming Partnerships

Forming product development partnerships (PDPs) around direct acting antivirals is a powerful means of rapidly discovering and developing antiviral therapeutics. PDPs are synergistic initiatives between academic innovators at the cutting edge of their discipline and biotechnology/pharma-based drug developers, who can rapidly move clinical development candidates forward through preclinical and into clinical development⁷⁹. Formation of these partnerships is absolutely necessary to maximize the possibility of success in a time and cost-efficient manner. Working with regulators during a pandemic is also important. Defining the resources that are needed and are already available is key. Both NCATS and NIAID have preclinical development resources that are readily accessible.

RESOURCES

RESOURCES

National Institutes of Health Accelerating COVID-19 Therapeutic Interventions and Vaccines (NIH ACTIV)

- In response to the emergence and rapid spread of the SARS-CoV-2 virus and of the Coronavirus Disease 2019 (COVID-19), NIH ACTIV brought together senior members of the National Institutes of Health (NIH), the biopharmaceutical industry, the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and academic researchers to establish a new biomedical research PPP to coordinate respond to COVID-19 and to plan for future pandemics.
- ACTIV working groups have been evaluating potential new therapeutics for preclinical and clinical development. Sponsors are encouraged to submitted agents to the therapeutic agent survey portal for expert review, guidance, and potential support for further efforts (e.g., inclusion in one of the ACTIV managed master protocols). (<https://redcap.ncats.nih.gov/redcap/surveys/index.php?s=DAE87WPTE7>)
- Description of the ACTIV clinical trial master protocols and additional resources to guide preclinical and clinical research on SARS-CoV2 can be found at the following link (<https://fnih.org/what-we-do/programs/activ-partnership>)

National Institute of Allergy and Infectious Diseases (NIAID)

- NIAID has a comprehensive suite of preclinical services available to the infectious disease research community to facilitate research in various stages in the product development pathway (www.niaid.nih.gov/research/microbiology-and-infectious-diseases-resources), including assays for testing candidate products against the SARS-CoV-2 in vitro and in animal models. Note that the purpose of these resources is not to assist researchers in developing a product from start to finish, but rather to lower the financial risk to product developers by providing limited, but critical, information to fill specific gaps in the product development pipeline.
- NIAID has many research resources available that may be of interest to you (<https://www.niaid.nih.gov/research/resources>), including SARS-CoV-2 reagents available from the BEI Resources Repository (<https://www.beiresources.org/>).

RESOURCES

National Institute of Allergy and Infectious Diseases (NIAID, continued)

- In addition to grants and contracts, NIAID also offers a range of basic, preclinical, and clinical resources for the scientific community to advance product development. Information on these services can be found at (<https://www.niaid.nih.gov/research/resources>). You do not need NIH funding to utilize these resources.
- The National Biocontainment Laboratories (NBLs) and Regional Biocontainment Laboratories (RBLs) provide BSL-4/3/2 and BSL-3/2 biocontainment facilities, respectively, for research on biodefense and emerging infectious disease agents. Investigators in academia, not-for-profit organizations, industry, and government studying biodefense and emerging infectious diseases may request the use of biocontainment laboratories. Please contact the NBLs and RBLs directly for further information.

National Center for Advancing Translational Sciences (NCATS)

- NCATS aims to address scientific and operational challenges that slow the development of new interventions to improve human health. Experts in NCATS's Division of Preclinical Innovation actively seek collaborators on various research projects, including SARS-CoV-2-related targets:
 - NCATS's early stage collaborative programs include 3-D Tissue model development, assay development (Assay Development and Screening Technology), high-throughput screening and hit-to-lead medicinal chemistry (Early Translation Branch), and informatics, as well as many others (<https://ncats.nih.gov/about/center/org/dpi/collaborate>).
 - NCATS staff can also provide expertise that enables and accelerates Investigational New Drug (IND) applications. Investigators or companies who have identified promising small molecules, biologics or gene therapies can form joint project teams with NCATS' Therapeutic Development Branch staff – including Bridging Interventional Development Gaps (BrIDGs) and Therapeutics for Rare and Neglected Diseases (TRND) scientists – to develop IND-ready therapies for consideration by the Food and Drug Administration for clinical testing.

RESOURCES

National Center for Advancing Translational Sciences (NCATS, continued)

- The NCATS COVID-19 OpenData Portal: NCATS has been generating a collection of datasets by screening a panel of SARS-CoV-2-related assays against ~10,000 annotated small molecules (including all approved drugs). These complete datasets, as well as the assay protocols used to generate them, are being made immediately available to the scientific community on the OpenData Portal as the screens are completed (<https://opendata.ncats.nih.gov/covid19>).
- In coordination with NCATS, and with support from the Foundation for the National Institutes of Health (FNIH), the National Institutes of Health (NIH) Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) Preclinical Working Group has been collecting and maintaining up-to-date summaries of COVID-19-related animal models, in both small animals and non-human primates (<https://opendata.ncats.nih.gov/covid19/animal>).

REFERENCES

REFERENCES

- (1) Hu, B.; Guo, H.; Zhou, P.; Shi, Z.-L. Characteristics of SARS-CoV-2 and COVID-19. *Nat. Rev. Microbiol.* 2020. <https://doi.org/10.1038/s41579-020-00459-7>.
- (2) Clercq, E. D. Molecular Targets for Antiviral Agents. *J. Pharmacol. Exp. Ther.* 2001, 297 (1), 1–10.
- (3) Shyr, Z. A.; Gorshkov, K.; Chen, C. Z.; Zheng, W. Drug Discovery Strategies for SARS-CoV-2. *J. Pharmacol. Exp. Ther.* 2020, 375 (1), 127–138. <https://doi.org/10.1124/jpet.120.000123>.
- (4) Arabi, Y. M.; Balkhy, H. H.; Hayden, F. G.; Bouchama, A.; Luke, T.; Baillie, J. K.; Al-Omari, A.; Hajeer, A. H.; Senga, M.; Denison, M. R.; Nguyen-Van-Tam, J. S.; Shindo, N.; Bermingham, A.; Chappell, J. D.; Van Kerkhove, M. D.; Fowler, R. A. Middle East Respiratory Syndrome <https://www.nejm.org/doi/full/10.1056/NEJMSr1408795> (accessed Dec 21, 2020). <https://doi.org/10.1056/NEJMSr1408795>.
- (5) Peiris, J. S. M.; Yuen, K. Y.; Osterhaus, A. D. M. E.; Stöhr, K. The Severe Acute Respiratory Syndrome <https://www.nejm.org/doi/10.1056/NEJMra032498> (accessed Dec 21, 2020). <https://doi.org/10.1056/NEJMra032498>.
- (6) Pardi, N.; Weissman, D. Development of Vaccines and Antivirals for Combating Viral Pandemics. *Nat. Biomed. Eng.* 2020, 4 (12), 1128–1133. <https://doi.org/10.1038/s41551-020-00658-w>.
- (7) Bar-On, Y. M.; Flamholz, A.; Phillips, R.; Milo, R. SARS-CoV-2 (COVID-19) by the Numbers. *eLife* 2020, 9, e57309. <https://doi.org/10.7554/eLife.57309>.
- (8) V'kovski, P.; Kratzel, A.; Steiner, S.; Stalder, H.; Thiel, V. Coronavirus Biology and Replication: Implications for SARS-CoV-2. *Nat. Rev. Microbiol.* 2020, 1–16. <https://doi.org/10.1038/s41579-020-00468-6>.
- (9) Yan, L.; Zhang, Y.; Ge, J.; Zheng, L.; Gao, Y.; Wang, T.; Jia, Z.; Wang, H.; Huang, Y.; Li, M.; Wang, Q.; Rao, Z.; Lou, Z. Architecture of a SARS-CoV-2 Mini Replication and Transcription Complex. *Nat. Commun.* 2020, 11 (1), 1–6. <https://doi.org/10.1038/s41467-020-19770-1>.
- (10) Robson, F.; Khan, K. S.; Le, T. K.; Paris, C.; Demirbag, S.; Barfuss, P.; Rocchi, P.; Ng, W.-L. Coronavirus RNA Proofreading: Molecular Basis and Therapeutic Targeting. *Mol. Cell* 2020, 79 (5), 710–727. <https://doi.org/10.1016/j.molcel.2020.07.027>.
- (11) De Clercq, E.; Li, G. Approved Antiviral Drugs over the Past 50 Years. *Clin. Microbiol. Rev.* 2016, 29 (3), 695–747. <https://doi.org/10.1128/CMR.00102-15>.
- (12) de Béthune, M.-P. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), Their Discovery, Development, and Use in the Treatment of HIV-1 Infection: A Review of the Last 20 Years (1989–2009). *Antiviral Res.* 2010, 85 (1), 75–90. <https://doi.org/10.1016/j.antiviral.2009.09.008>.
- (13) Eastman, R. T.; Roth, J. S.; Brimacombe, K. R.; Simeonov, A.; Shen, M.; Patnaik, S.; Hall, M. D. Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent. Sci.* 2020, 6 (5), 672. <https://doi.org/10.1021/acscentsci.0c00489>.

REFERENCES

- (14) Beigel, J. H.; Tomashek, K. M.; Dodd, L. E.; Mehta, A. K.; Zingman, B. S.; Kalil, A. C.; Hohmann, E.; Chu, H. Y.; Luetkemeyer, A.; Kline, S.; Lopez de Castilla, D.; Finberg, R. W.; Dierberg, K.; Tapson, V.; Hsieh, L.; Patterson, T. F.; Paredes, R.; Sweeney, D. A.; Short, W. R.; Touloumi, G.; Lye, D. C.; Ohmagari, N.; Oh, M.; Ruiz-Palacios, G. M.; Benfield, T.; Fätkenheuer, G.; Kortepeter, M. G.; Atmar, R. L.; Creech, C. B.; Lundgren, J.; Babiker, A. G.; Pett, S.; Neaton, J. D.; Burgess, T. H.; Bonnett, T.; Green, M.; Makowski, M.; Osinusi, A.; Nayak, S.; Lane, H. C. Remdesivir for the Treatment of Covid-19 – Final Report. *N. Engl. J. Med.* 2020, 383 (19), 1813–1826.
<https://doi.org/10.1056/NEJMoa2007764>.
- (15) Commissioner, O. of the. FDA Approves First Treatment for COVID-19
<https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19> (accessed Dec 21, 2020).
- (16) Sheahan, T. P.; Sims, A. C.; Zhou, S.; Graham, R. L.; Pruijssers, A. J.; Agostini, M. L.; Leist, S. R.; Schäfer, A.; Dinnon, K. H.; Stevens, L. J.; Chappell, J. D.; Lu, X.; Hughes, T. M.; George, A. S.; Hill, C. S.; Montgomery, S. A.; Brown, A. J.; Bluemling, G. R.; Natchus, M. G.; Saindane, M.; Kolykhalov, A. A.; Painter, G.; Harcourt, J.; Tamin, A.; Thornburg, N. J.; Swanstrom, R.; Denison, M. R.; Baric, R. S. An Orally Bioavailable Broad-Spectrum Antiviral Inhibits SARS-CoV-2 in Human Airway Epithelial Cell Cultures and Multiple Coronaviruses in Mice. *Sci. Transl. Med.* 2020, 12 (541), eabb5883. <https://doi.org/10.1126/scitranslmed.abb5883>.
- (17) Furuta, Y.; Komeno, T.; Nakamura, T. Favipiravir (T-705), a Broad Spectrum Inhibitor of Viral RNA Polymerase. *Proc. Jpn. Acad. Ser. B* 2017, 93 (7), 449–463. <https://doi.org/10.2183/pjab.93.027>.
- (18) Kaptein, S. J. F.; Jacobs, S.; Langendries, L.; Seldeslachts, L.; ter Horst, S.; Liesenborghs, L.; Hens, B.; Vergote, V.; Heylen, E.; Barthelemy, K.; Maas, E.; De Keyser, C.; Bervoets, L.; Rymenants, J.; Van Buyten, T.; Zhang, X.; Abdelnabi, R.; Pang, J.; Williams, R.; Thibaut, H. J.; Dallmeier, K.; Boudewijns, R.; Wouters, J.; Augustijns, P.; Verougstraete, N.; Cawthorne, C.; Breuer, J.; Solas, C.; Weynand, B.; Annaert, P.; Spriet, I.; Vande Velde, G.; Neyts, J.; Rocha-Pereira, J.; Delang, L. Favipiravir at High Doses Has Potent Antiviral Activity in SARS-CoV-2-infected Hamsters, Whereas Hydroxychloroquine Lacks Activity. *Proc. Natl. Acad. Sci.* 2020, 117 (43), 26955–26965.
<https://doi.org/10.1073/pnas.2014441117>.
- (19) Berliba, E.; Bogus, M.; Vanhoutte, F.; Berghmans, P.-J.; Good, S. S.; Moussa, A.; Pietropaolo, K.; Murphy, R. L.; Zhou, X.-J.; Sommadossi, J.-P. Safety, Pharmacokinetics, and Antiviral Activity of AT-527, a Novel Purine Nucleotide Prodrug, in Hepatitis C Virus-Infected Subjects with or without Cirrhosis. *Antimicrob. Agents Chemother.* 2019, 63 (12), e01201-19, /aac/63/12/AAC.01201-19.atom. <https://doi.org/10.1128/AAC.01201-19>.
- (20) Good, S. S.; Westover, J.; Jung, K.-H.; La Colla, P.; Collu, G.; Moussa, A.; Canard, B.; Sommadossi, J.-P. AT-527 Is a Potent in Vitro Replication Inhibitor of SARS-CoV-2, the Virus Responsible for the COVID-19 Pandemic; preprint, *Pharmacology and Toxicology*, 2020.
<https://doi.org/10.1101/2020.08.11.242834>.

REFERENCES

- (21) Taylor, R.; Kotian, P.; Warren, T.; Panchal, R.; Bavari, S.; Julander, J.; Dobo, S.; Rose, A.; El-Kattan, Y.; Taubenheim, B.; Babu, Y.; Sheridan, W. P. BCX4430 - A Broad-Spectrum Antiviral Adenosine Nucleoside Analog under Development for the Treatment of Ebola Virus Disease. *J. Infect. Public Health* 2016, 9 (3), 220–226. <https://doi.org/10.1016/j.jiph.2016.04.002>.
- (22) Tchesnokov, E. P.; Gordon, C. J.; Woolner, E.; Kocincova, D.; Perry, J. K.; Feng, J. Y.; Porter, D. P.; Gotte, M. Template-Dependent Inhibition of Coronavirus RNA-Dependent RNA Polymerase by Remdesivir Reveals a Second Mechanism of Action. *J. Biol. Chem.* 2020, jbc.AC120.015720. <https://doi.org/10.1074/jbc.AC120.015720>.
- (23) He, X.; Quan, S.; Xu, M.; Rodriguez, S.; Goh, S. L.; Wei, J.; Fridman, A.; Koeplinger, K. A.; Carroll, S. S.; Grobler, J. A.; Espeseth, A. S.; Olsen, D. B.; Hazuda, D. J.; Wang, D. Generation of a SARS-CoV-2 Replicon as a Model System to Dissect Virus Replication and Antiviral Inhibition; preprint; *Microbiology*, 2020. <https://doi.org/10.1101/2020.12.12.422532>.
- (24) Kotaki, T.; Xie, X.; Shi, P.-Y.; Kameoka, M. A PCR Amplicon-Based SARS-CoV-2 Replicon for Antiviral Screening; preprint; *Microbiology*, 2020. <https://doi.org/10.1101/2020.08.28.267567>.
- (25) Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.; Schiergens, T. S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; Müller, M. A.; Drosten, C.; Pöhlmann, S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020, 181 (2), 271-280.e8. <https://doi.org/10.1016/j.cell.2020.02.052>.
- (26) Johnson, B. A.; Xie, X.; Kalveram, B.; Lokugamage, K. G.; Muruato, A.; Zou, J.; Zhang, X.; Juelich, T.; Smith, J. K.; Zhang, L.; Bopp, N.; Schindewolf, C.; Vu, M.; Vanderheiden, A.; Swetnam, D.; Plante, J. A.; Aguilar, P.; Plante, K. S.; Lee, B.; Weaver, S. C.; Suthar, M. S.; Routh, A. L.; Ren, P.; Ku, Z.; An, Z.; Debbink, K.; Shi, P. Y.; Freiberg, A. N.; Menachery, V. D. Furin Cleavage Site Is Key to SARS-CoV-2 Pathogenesis; preprint; *Microbiology*, 2020. <https://doi.org/10.1101/2020.08.26.268854>.
- (27) Shrimp, J. H.; Kales, S. C.; Sanderson, P. E.; Simeonov, A.; Shen, M.; Hall, M. D. An Enzymatic TMPRSS2 Assay for Assessment of Clinical Candidates and Discovery of Inhibitors as Potential Treatment of COVID-19. *ACS Pharmacol. Transl. Sci.* 2020, 3 (5), 997–1007. <https://doi.org/10.1021/acsptsci.0c00106>.
- (28) Agbowuro, A. A.; Huston, W. M.; Gamble, A. B.; Tyndall, J. D. A. Proteases and Protease Inhibitors in Infectious Diseases. *Med. Res. Rev.* 2018, 38 (4), 1295–1331. <https://doi.org/10.1002/med.21475>.
- (29) Shin, D.; Mukherjee, R.; Grewe, D.; Bojkova, D.; Baek, K.; Bhattacharya, A.; Schulz, L.; Widera, M.; Mehdipour, A. R.; Tascher, G.; Geurink, P. P.; Wilhelm, A.; van der Heden van Noort, G. J.; Ovaa, H.; Müller, S.; Knobeloch, K.-P.; Rajalingam, K.; Schulman, B. A.; Cinatl, J.; Hummer, G.; Ciesek, S.; Dikic, I. Papain-like Protease Regulates SARS-CoV-2 Viral Spread and Innate Immunity. *Nature* 2020, 587 (7835), 657–662. <https://doi.org/10.1038/s41586-020-2601-5>.
- (30) Zhang, L.; Lin, D.; Sun, X.; Curth, U.; Drosten, C.; Sauerhering, L.; Becker, S.; Rox, K.; Hilgenfeld, R. Crystal Structure of SARS-CoV-2 Main Protease Provides a Basis for Design of Improved α -Ketoamide Inhibitors. *Science* 2020, 368 (6489), 409–412. <https://doi.org/10.1126/science.abb3405>.

REFERENCES

- (31) Boras, B.; Jones, R. M.; Anson, B. J.; Arenson, D.; Aschenbrenner, L.; Bakowski, M. A.; Beutler, N.; Binder, J.; Chen, E.; Eng, H.; Hammond, J.; Hoffman, R.; Kadar, E. P.; Kania, R.; Kimoto, E.; Kirkpatrick, M. G.; Lanyon, L.; Lendy, E. K.; Lillis, J. R.; Luthra, S. A.; Ma, C.; Noell, S.; Obach, R. S.; O'Brien, M. N.; O'Connor, R.; Ogilvie, K.; Owen, D.; Pettersson, M.; Reese, M. R.; Rogers, T. F.; Rossulek, M. I.; Sathish, J. G.; Steppan, C.; Ticehurst, M.; Updyke, L. W.; Zhu, Y.; Wang, J.; Chatterjee, A. K.; Mesecar, A. D.; Anderson, A. S.; Allerton, C. Discovery of a Novel Inhibitor of Coronavirus 3CL Protease as a Clinical Candidate for the Potential Treatment of COVID-19; preprint; *Pharmacology and Toxicology*, 2020. <https://doi.org/10.1101/2020.09.12.293498>.
- (32) Riva, L.; Yuan, S.; Yin, X.; Martin-Sancho, L.; Matsunaga, N.; Pache, L.; Burgstaller-Muehlbacher, S.; De Jesus, P. D.; Teriete, P.; Hull, M. V.; Chang, M. W.; Chan, J. F.-W.; Cao, J.; Poon, V. K.-M.; Herbert, K. M.; Cheng, K.; Nguyen, T.-T. H.; Rubanov, A.; Pu, Y.; Nguyen, C.; Choi, A.; Rathnasinghe, R.; Schotsaert, M.; Miorin, L.; Dejoze, M.; Zwaka, T. P.; Sit, K.-Y.; Martinez-Sobrido, L.; Liu, W.-C.; White, K. M.; Chapman, M. E.; Lendy, E. K.; Glynne, R. J.; Albrecht, R.; Rupp, E.; Mesecar, A. D.; Johnson, J. R.; Benner, C.; Sun, R.; Schultz, P. G.; Su, A. I.; García-Sastre, A.; Chatterjee, A. K.; Yuen, K.-Y.; Chanda, S. K. Discovery of SARS-CoV-2 Antiviral Drugs through Large-Scale Compound Repurposing. *Nature* 2020, 586 (7827), 113–119. <https://doi.org/10.1038/s41586-020-2577-1>.
- (33) Ghosh, A. K.; Brindisi, M.; Shahabi, D.; Chapman, M. E.; Mesecar, A. D. Drug Development and Medicinal Chemistry Efforts toward SARS-Coronavirus and Covid-19 Therapeutics. *ChemMedChem* 2020, 15 (11), 907–932. <https://doi.org/10.1002/cmdc.202000223>.
- (34) Clasman, J. R.; Báez-Santos, Y. M.; Mettelman, R. C.; O'Brien, A.; Baker, S. C.; Mesecar, A. D. X-Ray Structure and Enzymatic Activity Profile of a Core Papain-like Protease of MERS Coronavirus with Utility for Structure-Based Drug Design. *Sci. Rep.* 2017, 7 (1), 40292. <https://doi.org/10.1038/srep40292>.
- (35) Daczkowski, C. M.; Dzimianski, J. V.; Clasman, J. R.; Goodwin, O.; Mesecar, A. D.; Pegan, S. D. Structural Insights into the Interaction of Coronavirus Papain-Like Proteases and Interferon-Stimulated Gene Product 15 from Different Species. *J. Mol. Biol.* 2017, 429 (11), 1661–1683. <https://doi.org/10.1016/j.jmb.2017.04.011>.
- (36) Henes, M.; Lockbaum, G. J.; Kosovrasti, K.; Leidner, F.; Nachum, G. S.; Nalivaika, E. A.; Lee, S.-K.; Spielvogel, E.; Zhou, S.; Swanstrom, R.; Bolon, D. N. A.; Kurt Yilmaz, N.; Schiffer, C. A. Picomolar to Micromolar: Elucidating the Role of Distal Mutations in HIV-1 Protease in Conferring Drug Resistance. *ACS Chem. Biol.* 2019, 14 (11), 2441–2452. <https://doi.org/10.1021/acscchembio.9b00370>.
- (37) Leidner, F.; Kurt-Yilmaz, N.; Schiffer, C. A. Deciphering Complex Mechanisms of Resistance and Loss of Potency through Coupled Molecular Dynamics and Machine Learning; preprint; *Biophysics*, 2020. <https://doi.org/10.1101/2020.06.08.139105>.

REFERENCES

- (38) Spielvogel, E.; Lee, S.-K.; Zhou, S.; Lockbaum, G. J.; Henes, M.; Sondgeroth, A.; Kosovrasti, K.; Ali, A.; Yilmaz, N. K.; Schiffer, C. A.; Swanstrom, R. Selection of HIV-1 for Resistance to Fourth Generation Protease Inhibitors Reveals Two Independent Pathways to High-Level Resistance; preprint; *Microbiology*, 2019. <https://doi.org/10.1101/837120>.
- (39) Kurt Yilmaz, N.; Swanstrom, R.; Schiffer, C. A. Improving Viral Protease Inhibitors to Counter Drug Resistance. *Trends Microbiol.* 2016, 24 (7), 547–557. <https://doi.org/10.1016/j.tim.2016.03.010>.
- (40) Matthew, A. N.; Zephyr, J.; Nageswara Rao, D.; Henes, M.; Kamran, W.; Kosovrasti, K.; Hedger, A. K.; Lockbaum, G. J.; Timm, J.; Ali, A.; Kurt Yilmaz, N.; Schiffer, C. A. Avoiding Drug Resistance by Substrate Envelope-Guided Design: Toward Potent and Robust HCV NS3/4A Protease Inhibitors. *mBio* 2020, 11 (2), e00172-20, /mbio/11/2/mBio.00172-20.atom. <https://doi.org/10.1128/mBio.00172-20>.
- (41) Haniff, H. S.; Tong, Y.; Liu, X.; Chen, J. L.; Suresh, B. M.; Andrews, R. J.; Peterson, J. M.; O’Leary, C. A.; Benhamou, R. I.; Moss, W. N.; Disney, M. D. Targeting the SARS-CoV-2 RNA Genome with Small Molecule Binders and Ribonuclease Targeting Chimera (RIBOTAC) Degradable. *ACS Cent. Sci.* 2020, 6 (10), 1713–1721. <https://doi.org/10.1021/acscentsci.0c00984>.
- (42) Cao, L.; Goureshnik, I.; Coventry, B.; Case, J. B.; Miller, L.; Kozodoy, L.; Chen, R. E.; Carter, L.; Walls, A. C.; Park, Y.-J.; Strauch, E.-M.; Stewart, L.; Diamond, M. S.; Velesler, D.; Baker, D. De Novo Design of Picomolar SARS-CoV-2 Miniprotein Inhibitors. *Science* 2020, 370 (6515), 426–431. <https://doi.org/10.1126/science.abd9909>.
- (43) Gordon, D. E. et al. A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing. *Nature* 2020, 583 (7816), 459–468. <https://doi.org/10.1038/s41586-020-2286-9>.
- (44) Bojkova, D.; Klann, K.; Koch, B.; Widera, M.; Krause, D.; Ciesek, S.; Cinatl, J.; Münch, C. Proteomics of SARS-CoV-2-Infected Host Cells Reveals Therapy Targets. *Nature* 2020, 583 (7816), 469–472. <https://doi.org/10.1038/s41586-020-2332-7>.
- (45) Dittmar, M.; Lee, J. S.; Whig, K.; Segrist, E.; Li, M.; Jurado, K.; Samby, K.; Ramage, H.; Schultz, D.; Cherry, S. Drug Repurposing Screens Reveal FDA Approved Drugs Active against SARS-Cov-2; preprint; *Microbiology*, 2020. <https://doi.org/10.1101/2020.06.19.161042>.
- (46) Ellinger, B.; Bojkova, D.; Zaliani, A.; Cinatl, J.; Claussen, C.; Westhaus, S.; Reinshagen, J.; Kuzikov, M.; Wolf, M.; Geisslinger, G.; Gribbon, P.; Ciesek, S. Identification of Inhibitors of SARS-CoV-2 in-Vitro Cellular Toxicity in Human (Caco-2) Cells Using a Large Scale Drug Repurposing Collection; preprint; In Review, 2020. <https://doi.org/10.21203/rs.3.rs-23951/v1>.
- (47) Jeon, S.; Ko, M.; Lee, J.; Choi, I.; Byun, S. Y.; Park, S.; Shum, D.; Kim, S. Identification of Antiviral Drug Candidates against SARS-CoV-2 from FDA-Approved Drugs. *Antimicrob. Agents Chemother.* 2020, 64 (7), e00819-20, /aac/64/7/AAC.00819-20.atom. <https://doi.org/10.1128/AAC.00819-20>.

REFERENCES

- (48) Ko, M.; Jeon, S.; Ryu, W.; Kim, S. Comparative Analysis of Antiviral Efficacy of FDA-approved Drugs against SARS-CoV-2 in Human Lung Cells. *J. Med. Virol.* 2020, jmv.26397. <https://doi.org/10.1002/jmv.26397>.
- (49) Touret, F.; Gilles, M.; Barral, K.; Nougairède, A.; van Helden, J.; Decroly, E.; de Lamballerie, X.; Coutard, B. In Vitro Screening of a FDA Approved Chemical Library Reveals Potential Inhibitors of SARS-CoV-2 Replication. *Sci. Rep.* 2020, 10 (1), 13093. <https://doi.org/10.1038/s41598-020-70143-6>.
- (50) Schor, S.; Einav, S. Repurposing of Kinase Inhibitors as Broad-Spectrum Antiviral Drugs. *DNA Cell Biol.* 2018, 37 (2), 63–69. <https://doi.org/10.1089/dna.2017.4033>.
- (51) Goodwin, C. M.; Xu, S.; Munger, J. Stealing the Keys to the Kitchen: Viral Manipulation of the Host Cell Metabolic Network. *Trends Microbiol.* 2015, 23 (12), 789–798. <https://doi.org/10.1016/j.tim.2015.08.007>.
- (52) Koyuncu, E.; Budayeva, H. G.; Miteva, Y. V.; Ricci, D. P.; Silhavy, T. J.; Shenk, T.; Cristea, I. M. Sirtuins Are Evolutionarily Conserved Viral Restriction Factors. *mBio* 2014, 5 (6), e02249-14. <https://doi.org/10.1128/mBio.02249-14>.
- (53) Acosta, E.; Bowlin, T.; Brooks, J.; Chiang, L.; Hussein, I.; Kimberlin, D.; Kauvar, L. M.; Leavitt, R.; Prichard, M.; Whitley, R. Advances in the Development of Therapeutics for Cytomegalovirus Infections. *J. Infect. Dis.* 2020, 221 (Supplement_1), S32–S44. <https://doi.org/10.1093/infdis/jiz493>.
- (54) Xie, X.; Muruato, A. E.; Zhang, X.; Lokugamage, K. G.; Fontes-Garfias, C. R.; Zou, J.; Liu, J.; Ren, P.; Balakrishnan, M.; Cihlar, T.; Tseng, C.-T. K.; Makino, S.; Menachery, V. D.; Bilello, J. P.; Shi, P.-Y. A Nanoluciferase SARS-CoV-2 for Rapid Neutralization Testing and Screening of Anti-Infective Drugs for COVID-19. *Nat. Commun.* 2020, 11 (1), 5214. <https://doi.org/10.1038/s41467-020-19055-7>.
- (55) Bobrowski, T.; Chen, L.; Eastman, R. T.; Itkin, Z.; Shinn, P.; Chen, C. Z.; Guo, H.; Zheng, W.; Michael, S.; Simeonov, A.; Hall, M. D.; Zakharov, A. V.; Muratov, E. N. Synergistic and Antagonistic Drug Combinations against SARS-CoV-2. *Mol. Ther.* 2020, S1525001620306730. <https://doi.org/10.1016/j.ymthe.2020.12.016>.
- (56) Pruijssers, A. J.; George, A. S.; Schäfer, A.; Leist, S. R.; Gralinski, L. E.; Dinnon, K. H.; Yount, B. L.; Agostini, M. L.; Stevens, L. J.; Chappell, J. D.; Lu, X.; Hughes, T. M.; Gully, K.; Martinez, D. R.; Brown, A. J.; Graham, R. L.; Perry, J. K.; Du Pont, V.; Pitts, J.; Ma, B.; Babusis, D.; Murakami, E.; Feng, J. Y.; Bilello, J. P.; Porter, D. P.; Cihlar, T.; Baric, R. S.; Denison, M. R.; Sheahan, T. P. Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV Expressing the SARS-CoV-2 RNA Polymerase in Mice. *Cell Rep.* 2020, 32 (3), 107940. <https://doi.org/10.1016/j.celrep.2020.107940>.

REFERENCES

- (57) Funnell, S. G. P.; Dowling, W. E.; Muñoz-Fontela, C.; Gsell, P.-S.; Ingber, D. E.; Hamilton, G. A.; Delang, L.; Rocha-Pereira, J.; Kaptein, S.; Dallmeier, K. H.; Neyts, J.; Rosenke, K.; de Wit, E.; Feldmann, H.; Maisonnasse, P.; Le Grand, R.; Frieman, M. B.; Coleman, C. M. Emerging Preclinical Evidence Does Not Support Broad Use of Hydroxychloroquine in COVID-19 Patients. *Nat. Commun.* 2020, 11 (1), 4253. <https://doi.org/10.1038/s41467-020-17907-w>.
- (58) Xie, X.; Muruato, A.; Lokugamage, K. G.; Narayanan, K.; Zhang, X.; Zou, J.; Liu, J.; Schindewolf, C.; Bopp, N. E.; Aguilar, P. V.; Plante, K. S.; Weaver, S. C.; Makino, S.; LeDuc, J. W.; Menachery, V. D.; Shi, P.-Y. An Infectious CDNA Clone of SARS-CoV-2. *Cell Host Microbe* 2020, 27 (5), 841-848.e3. <https://doi.org/10.1016/j.chom.2020.04.004>.
- (59) Hou, Y. J.; Okuda, K.; Edwards, C. E.; Martinez, D. R.; Asakura, T.; Dinnon, K. H.; Kato, T.; Lee, R. E.; Yount, B. L.; Mascenik, T. M.; Chen, G.; Olivier, K. N.; Ghio, A.; Tse, L. V.; Leist, S. R.; Gralinski, L. E.; Schäfer, A.; Dang, H.; Gilmore, R.; Nakano, S.; Sun, L.; Fulcher, M. L.; Livraghi-Butrico, A.; Nicely, N. I.; Cameron, M.; Cameron, C.; Kelvin, D. J.; de Silva, A.; Margolis, D. M.; Markmann, A.; Bartelt, L.; Zumwalt, R.; Martinez, F. J.; Salvatore, S. P.; Borczuk, A.; Tata, P. R.; Sontake, V.; Kimple, A.; Jaspers, I.; O'Neal, W. K.; Randell, S. H.; Boucher, R. C.; Baric, R. S. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. *Cell* 2020, 182 (2), 429-446.e14. <https://doi.org/10.1016/j.cell.2020.05.042>.
- (60) Hewitt, J. A.; Lutz, C.; Florence, W. C.; Pitt, M. L. M.; Rao, S.; Rappaport, J.; Haigwood, N. L. ACTIVating Resources for the COVID-19 Pandemic: In Vivo Models for Vaccines and Therapeutics. *Cell Host Microbe* 2020, 28 (5), 646–659. <https://doi.org/10.1016/j.chom.2020.09.016>.
- (61) Animal Models <https://opendata.ncats.nih.gov/covid19/animal> (accessed Dec 21, 2020).
- (62) Shi, J.; Wen, Z.; Zhong, G.; Yang, H.; Wang, C.; Huang, B.; Liu, R.; He, X.; Shuai, L.; Sun, Z.; Zhao, Y.; Liu, P.; Liang, L.; Cui, P.; Wang, J.; Zhang, X.; Guan, Y.; Tan, W.; Wu, G.; Chen, H.; Bu, Z. Susceptibility of Ferrets, Cats, Dogs, and Other Domesticated Animals to SARS–Coronavirus 2. *Science* 2020, 368 (6494), 1016–1020. <https://doi.org/10.1126/science.abb7015>.
- (63) Hassan, A. O.; Case, J. B.; Winkler, E. S.; Thackray, L. B.; Kafai, N. M.; Bailey, A. L.; McCune, B. T.; Fox, J. M.; Chen, R. E.; Alsoussi, W. B.; Turner, J. S.; Schmitz, A. J.; Lei, T.; Shrihari, S.; Keeler, S. P.; Fremont, D. H.; Greco, S.; McCray, P. B.; Perlman, S.; Holtzman, M. J.; Ellebedy, A. H.; Diamond, M. S. A SARS-CoV-2 Infection Model in Mice Demonstrates Protection by Neutralizing Antibodies. *Cell* 2020, 182 (3), 744-753.e4. <https://doi.org/10.1016/j.cell.2020.06.011>.
- (64) Dinnon, K. H.; Leist, S. R.; Schäfer, A.; Edwards, C. E.; Martinez, D. R.; Montgomery, S. A.; West, A.; Yount, B. L.; Hou, Y. J.; Adams, L. E.; Gully, K. L.; Brown, A. J.; Huang, E.; Bryant, M. D.; Choong, I. C.; Glenn, J. S.; Gralinski, L. E.; Sheahan, T. P.; Baric, R. S. A Mouse-Adapted Model of SARS-CoV-2 to Test COVID-19 Countermeasures. *Nature* 2020, 586 (7830), 560–566. <https://doi.org/10.1038/s41586-020-2708-8>.

REFERENCES

- (65) Chan, J. F.-W.; Zhang, A. J.; Yuan, S.; Poon, V. K.-M.; Chan, C. C.-S.; Lee, A. C.-Y.; Chan, W.-M.; Fan, Z.; Tsoi, H.-W.; Wen, L.; Liang, R.; Cao, J.; Chen, Y.; Tang, K.; Luo, C.; Cai, J.-P.; Kok, K.-H.; Chu, H.; Chan, K.-H.; Sridhar, S.; Chen, Z.; Chen, H.; To, K. K.-W.; Yuen, K.-Y. Simulation of the Clinical and Pathological Manifestations of Coronavirus Disease 2019 (COVID-19) in a Golden Syrian Hamster Model: Implications for Disease Pathogenesis and Transmissibility. *Clin. Infect. Dis.* 2020, ciaa325. <https://doi.org/10.1093/cid/ciaa325>.
- (66) Osterrieder, N.; Bertzbach, L. D.; Dietert, K.; Abdelgawad, A.; Vladimirova, D.; Kunec, D.; Hoffmann, D.; Beer, M.; Gruber, A. D.; Trimpert, J. Age-Dependent Progression of SARS-CoV-2 Infection in Syrian Hamsters. *Viruses* 2020, 12 (7), 779. <https://doi.org/10.3390/v12070779>.
- (67) Kim, Y.-I.; Kim, S.-G.; Kim, S.-M.; Kim, E.-H.; Park, S.-J.; Yu, K.-M.; Chang, J.-H.; Kim, E. J.; Lee, S.; Casel, M. A. B.; Um, J.; Song, M.-S.; Jeong, H. W.; Lai, V. D.; Kim, Y.; Chin, B. S.; Park, J.-S.; Chung, K.-H.; Foo, S.-S.; Poo, H.; Mo, I.-P.; Lee, O.-J.; Webby, R. J.; Jung, J. U.; Choi, Y. K. Infection and Rapid Transmission of SARS-CoV-2 in Ferrets. *Cell Host Microbe* 2020, 27 (5), 704-709.e2. <https://doi.org/10.1016/j.chom.2020.03.023>.
- (68) Munster, V. J.; Feldmann, F.; Williamson, B. N.; van Doremalen, N.; Pérez-Pérez, L.; Schulz, J.; Meade-White, K.; Okumura, A.; Callison, J.; Brumbaugh, B.; Avanzato, V. A.; Rosenke, R.; Hanley, P. W.; Saturday, G.; Scott, D.; Fischer, E. R.; de Wit, E. Respiratory Disease in Rhesus Macaques Inoculated with SARS-CoV-2. *Nature* 2020, 585 (7824), 268–272. <https://doi.org/10.1038/s41586-020-2324-7>.
- (69) Williamson, B. N.; Feldmann, F.; Schwarz, B.; Meade-White, K.; Porter, D. P.; Schulz, J.; Doremalen, N. van; Leighton, I.; Yinda, C. K.; Pérez-Pérez, L.; Okumura, A.; Lovaglio, J.; Hanley, P. W.; Saturday, G.; Bosio, C. M.; Anzick, S.; Barbian, K.; Cihlar, T.; Martens, C.; Scott, D. P.; Munster, V. J.; Wit, E. de. Clinical Benefit of Remdesivir in Rhesus Macaques Infected with SARS-CoV-2; preprint; *Microbiology*, 2020. <https://doi.org/10.1101/2020.04.15.043166>.
- (70) Lamb, Y. N. Remdesivir: First Approval. *Drugs* 2020, 80 (13), 1355–1363. <https://doi.org/10.1007/s40265-020-01378-w>.
- (71) Chen, M.; Shen, W.; Rowan, N. R.; Kulaga, H.; Hillel, A.; Ramanathan, M.; Lane, A. P. Elevated ACE-2 Expression in the Olfactory Neuroepithelium: Implications for Anosmia and Upper Respiratory SARS-CoV-2 Entry and Replication. *Eur. Respir. J.* 2020, 56 (3), 2001948. <https://doi.org/10.1183/13993003.01948-2020>.
- (72) Seley-Radtke, K. L.; Yates, M. K. The Evolution of Nucleoside Analogue Antivirals: A Review for Chemists and Non-Chemists. Part I: Early Structural Modifications to the Nucleoside Scaffold. *Antiviral Res.* 2018, 154, 66–86. <https://doi.org/10.1016/j.antiviral.2018.04.004>.
- (73) Yates, M. K.; Seley-Radtke, K. L. The Evolution of Antiviral Nucleoside Analogues: A Review for Chemists and Non-Chemists. Part II: Complex Modifications to the Nucleoside Scaffold. *Antiviral Res.* 2019, 162, 5–21. <https://doi.org/10.1016/j.antiviral.2018.11.016>.

REFERENCES

- (74) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. Advances in the Development of Nucleoside and Nucleotide Analogues for Cancer and Viral Diseases. *Nat. Rev. Drug Discov.* 2013, 12 (6), 447–464. <https://doi.org/10.1038/nrd4010>.
- (75) Villas-Boas, G. R.; Rescia, V. C.; Paes, M. M.; Lavorato, S. N.; Magalhães-Filho, M. F. de; Cunha, M. S.; Simões, R. da C.; Lacerda, R. B. de; Freitas-Júnior, R. S. de; Ramos, B. H. da S.; Mapeli, A. M.; Henriques, M. da S. T.; Freitas, W. R. de; Lopes, L. A. F.; Oliveira, L. G. R.; Silva, J. G. da; Silva-Filho, S. E.; Silveira, A. P. S. da; Leão, K. V.; Matos, M. M. de S.; Fernandes, J. S.; Cuman, R. K. N.; Silva-Comar, F. M. de S.; Comar, J. F.; Brasileiro, L. do A.; Santos, J. N. dos; Oesterreich, S. A. The New Coronavirus (SARS-CoV-2): A Comprehensive Review on Immunity and the Application of Bioinformatics and Molecular Modeling to the Discovery of Potential Anti-SARS-CoV-2 Agents. *Molecules* 2020, 25 (18), 4086. <https://doi.org/10.3390/molecules25184086>.
- (76) Zhang, D.; Luo, G.; Ding, X.; Lu, C. Preclinical Experimental Models of Drug Metabolism and Disposition in Drug Discovery and Development. *Acta Pharm. Sin. B* 2012, 2 (6), 549–561. <https://doi.org/10.1016/j.apsb.2012.10.004>.
- (77) Boda, B.; Benaoudia, S.; Huang, S.; Bonfante, R.; Wiszniewski, L.; Tseligka, E. D.; Tapparell, C.; Constant, S. Antiviral Drug Screening by Assessing Epithelial Functions and Innate Immune Responses in Human 3D Airway Epithelium Model. *Antiviral Res.* 2018, 156, 72–79. <https://doi.org/10.1016/j.antiviral.2018.06.007>.
- (78) Research, C. for D. E. and. Antiviral Product Development--Conducting and Submitting Virology Studies to the Agency <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/antiviral-product-development-conducting-and-submitting-virology-studies-agency> (accessed Dec 21, 2020).
- (79) Everts, M.; Cihlar, T.; Bostwick, J. R.; Whitley, R. J. Accelerating Drug Development: Antiviral Therapies for Emerging Viruses as a Model. *Annu. Rev. Pharmacol. Toxicol.* 2017, 57 (1), 155–169. <https://doi.org/10.1146/annurev-pharmtox-010716-104533>.

