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Experimental Models of SARS-CoV-2 Infection: Possible Platforms to Study COVID-19 Pathogenesis and Potential Treatments

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Abstract

In December 2019, a novel coronavirus crossed species barriers to infect humans and was effectively transmitted from person to person, leading to a worldwide pandemic. Development of effective clinical interventions,

including vaccines and antiviral drugs that could prevent or limit the burden or transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global health priority. It is thus of utmost importance to assess possible therapeutic strategies against SARS-CoV-2 using experimental models that recapitulate aspects of the human disease. Here, we review available models currently being developed and used to study SARS-CoV-2 infection and highlight their application to screen potential therapeutic approaches, including repurposed antiviral drugs and vaccines. Each identified model provides a valuable insight into SARS-CoV-2 cellular tropism, replication kinetics, and cell damage that could ultimately enhance understanding of SARS-CoV-2 pathogenesis and protective immunity.

1. INTRODUCTION

In December 2019 in Wuhan, China, a novel coronavirus outbreak occurred that caused infectious respiratory disease. Soon after, due to its high transmissibility and pathogenicity, the virus spread across international borders. The World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a pandemic, which has become the worst public health crisis in a century. In February 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative agent of COVID-19, and as of November 10, 2020, it has infected more than 50.6 million people worldwide and caused more than 1.26 million recorded deaths. Similar to other lethal coronaviruses [i.e., severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)], SARS-CoV-2 triggers an excessive immune response known as a cytokine storm that can lead to multiple organ failure and death (1).

SARS-CoV-2 is a single-stranded RNA virus. Its envelope is covered with trimeric spike (S) proteins that bind to human angiotensin-converting enzyme 2 (hACE2) on the plasma membrane of host cells (2). After binding, the S protein is cleaved by the transmembrane protease serine subtype 2 (TMPRSS2), thereby facilitating viral entry (3). Following fusion of viral and cellular membranes, the virus empties its genetic contents and hijacks the translational machinery of the host cells to perpetuate itself, resulting in the production of copies of the virus at the expense of the host cells.

The main challenge in the current pandemic is the novelty of the disease, which results in unfamiliar pathophysiology and limited knowledge of SARS-CoV-2 biology (4). At the time this manuscript was submitted for publication, there were three COVID-19 vaccines for which certain national regulatory authorities had authorized the use. There are also many other potential COVID-19 vaccine candidates currently in development, and several of these are likely to be approved by the time of this publication. Nevertheless, for this pandemic and for others that will inevitably occur in the near future, it is of utmost importance to use platforms with physiologically relevant model systems that can faithfully indicate the viral life cycle, delineate the pathology, recapitulate host-virus interactions in human cells, and provide a platform to test protective treatments and therapies such as potential vaccines and antiviral drugs. Models of SARS-CoV-2 infection are also useful for evaluating other emerging strategies, for example, stem cell therapies that might combat COVID-19 (5). In this review, we summarize several model systems that have recently been used to study SARS-CoV-2 (**Figure 1**). Here, we attempt to define the hierarchy in the use of model organisms, from simple monolayer cell culture platforms to complex tissue explant cultures in Section 2. We also discuss animal models ranging from mouse models to nonhuman primates (NHPs). Please note that the authors do not include experimental models identified after November 10, 2020.

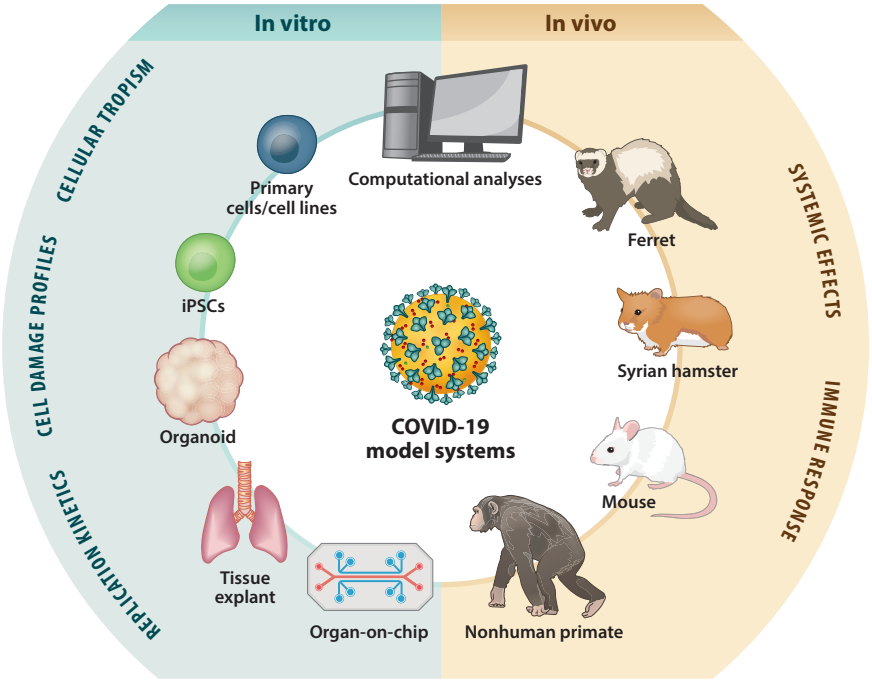

Figure 1

Overview of different model systems used to study severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. A wide range of in vitro models, including primary cell culture, cell lines, induced pluripotent stem cell (iPSC)-derived stem cells, organoids, tissue explants, and organ-on-a-chip, have been used as platforms to study SARS-CoV-2 replication kinetics, cell damage profiles, and cellular tropism. Furthermore, animal models in mice, ferrets, Syrian hamsters, and nonhuman primates are used to evaluate SARS-CoV-2 infection in specific target organs. These platforms are also useful to assess systemic effects and immune responses. In addition, numerous computational and mathematical models can predict trends of coronavirus disease 2019 (COVID-19) transmission and the number of infected individuals, thereby aiding in epidemic prevention and control measures.

2. IN VITRO MODELS

As with SARS-CoV, MERS-CoV, and other emerging viruses, in vitro models can yield understanding of viral replication kinetics and the profile of viral-induced cell damage. Studies of SARS-CoV-2 have involved many in vitro models: primary cell culture, cell lines, induced pluripotent stem cell (iPSC)-derived cells, organ-on-a-chip, organoids, and tissue explants (**Table 1**).

Although SARS-CoV-2 primarily targets lung epithelium and causes respiratory infection, growing evidence documents that the intestine, kidney, liver, pancreas, brain, heart, blood vessels, and other organs can also be infected. Accordingly, SARS-CoV-2 infection has been assessed in numerous in vitro platforms derived from different human tissues and organs (**Table 2**).

2.1. Primary Cell Culture

Based on their high expression of angiotensin-converting enzyme 2 (ACE2) and TMPRSS2, human airway epithelial cells such as nasal (goblet and ciliated cells) and bronchial cells provide clinically relevant models for screening drugs against SARS-CoV-2 (6). Pizzorno et al. (7) used human airway epithelium (HAE) cultured at an air-liquid interface (ALI) to characterize viral

Table 1 Benefits and limitations of in vitro models in COVID-19 research

Experimental model	Benefits (references)	Limitation(s) (references)
Primary cell culture	Reproduces major structural features of human respiratory epithelia (7) Can test immune modulators and antivirals for SARS-CoV-2 infection (8)	Availability and capacity for expansion are limited Absence of immune cells (7)
Cell lines	Suitable to replicate and isolate SARS-CoV-2 (13, 14) Useful for characterizing cellular tropism, viral replication kinetics, and virus-induced cell damage profile of SARS-CoV-2 (21)	Cell line tropism might not fully represent how SARS-CoV-2 replicates and affects human organs in the physiological state (21) Constant proliferation and lack of polarization might impact the transfection efficiency of SARS-CoV-2 (24)
iPSC-derived cells	Transcriptome of iPSC-derived cells is similar to their respective primary counterparts and allows them to respond to immune stimuli (26) Useful for drug repurposing (25)	Directed differentiation of hiPSCs to desired cell types is time consuming Cellular immaturity of hiPSC-derived cells can affect their susceptibility to SARS-CoV-2 (30)
Organoids	Contain a full range of differentiated cell types as are present in the target organ Aid in understanding the tissue tropism of SARS-CoV-2 (24, 54, 57) Useful tools for antiviral drug discovery and development (55)	Typically lack vasculature and immune cells (184)
Organ-on-a-chip	Provides vascular perfusion, organ-level physical microenvironments, tissue-tissue interfaces, relevant mechanical cues, and fluid flow Human lung airway chip may be useful to expedite drug repurposing (61)	Relatively low throughput
Tissue explant	Retains cytoarchitecture and three-dimensional organization of the tissue Provides a suitable model to study tropism, replication competence, and innate immune responses of SARS-CoV-2 in the human respiratory tract (21, 68)	Ex vivo tissue explant culture is short lasting Lacks in vivo effects of host adaptive immune and systemic inflammatory responses (68) Supply of human tissue is difficult

Abbreviations: COVID-19, coronavirus disease 2019; hiPSC, human induced pluripotent stem cell; iPSC, induced pluripotent stem cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

infection kinetics, tissue-level remodeling of the cellular ultrastructure, and transcriptional immune signatures induced by SARS-CoV-2. Since this model can closely recapitulate virus-host cell interactions, as in infected upper and lower airways, it has been used to evaluate the efficacy of antiviral drugs (8, 9). Primary cultures mainly characterize short-lived SARS-CoV-2 infection in HAE, but Hao et al. (10) reported data for SARS-CoV-2 in polarized HAE and ALI culture that allowed for the monitoring of SARS-CoV-2 infections for a prolonged time period. Another group used conditional cell reprogramming technology to investigate long-term cultures of cells from the human upper and lower respiratory tract (11).

2.2. Cell Lines

As with the use of cell culture models for characterizing cell tropism, virus-induced cell damage, and viral replication of emerging viruses, a set of cell lines have been utilized to study

Table 2 In vitro models currently used in COVID-19 research

Model type (Origin)	Application(s)	Reference(s)
Primary cell culture		
Human airway epithelium	Characterizing viral infection kinetics and tissue-level remodeling of cellular ultrastructure and transcriptional immune signatures induced by SARS-CoV-2	7
	Drug screening	8
	Understanding responses of proximal and distal lung epithelium to SARS-CoV-2 infection	8, 9
	Long-term modeling of SARS-CoV-2 infection in polarized human airway epithelium	10
	Addressing SARS-CoV-2 replication	185
Cell lines		
VeroE6 cells, wild type (kidney epithelial cells of African green monkey)	Replicating and isolating SARS-CoV-2	3, 14, 15
VeroE6 cells, engineered to over-express TMPRSS2 or VeroE6/TMPRSS2 (kidney epithelial cells of African green monkey)	Enhanced isolation of SARS-CoV-2	13
Caco-2 cells (human epithelial colorectal adenocarcinoma)	Screening 5,632 compounds for their potential to inhibit SARS-CoV-2-induced cytotoxicity	16
	Revealing central pathways relevant for SARS-CoV-2 infection	17
Calu-3 cells (human lung adenocarcinoma)	Defining SARS-CoV-2 replication kinetics	13, 18, 20
	Drug screening (evaluating the inhibitory effect of chloroquine)	19
A549 cells (human lung adenocarcinoma)	Comparing tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV	21
	Studying SARS-CoV-2 replication	15, 186
	Evaluating the expression of ACE2 and TMPRSS2	187
Huh7 cells (hepatocellular carcinoma)	Comparing tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV	21
	Drug screening	193
	Defining the cellular response to the virus	188
HeLa cells (human cervical adenocarcinoma)	Determining SARS-CoV-2 cell entry mechanisms	2, 189, 194
293T cells (human embryonic kidney)	Evaluating the inhibitory activity of EK1C4 against SARS-CoV-2	190
	Assessing syncytia formation upon SARS-CoV-2 infection	191
	Testing human monoclonal antibody blockade of SARS-CoV-2 infection	192
U251 cells (human brain glioblastoma)	Comparing tropism, replication kinetics, and cell damage by SARS-CoV-2 and SARS-CoV	21
RD cells (human rhabdomyosarcoma)	Comparing tropism, replication kinetics, and cell damage by SARS-CoV-2 and SARS-CoV	21
	Evaluating the inhibitory activity of EK1C4 against SARS-CoV-2	190

(*Continued*)

Table 2 (*Continued*)

Model type (Origin)	Application(s)	Reference(s)
Human iPSC–derived stem cells		
iPSC-derived lung epithelium	Modeling SARS-CoV-2 infection and drug repurposing	25
iPSC-derived alveolar epithelial type 2 cells	Studying transcriptomic alteration after SARS-CoV-2 infection and drug screening	26
	Studying expression level of genes relevant for COVID-19 disease modeling	27
iPSC-derived airway epithelial cells	Modeling SARS-CoV-2 infection	27
iPSC-derived lung and macrophage coculture	Modeling host-pathogen interaction and immune response caused by SARS-CoV-2 infection	28
iPSC-derived cardiomyocytes	Revealing that cardiomyocytes are permissive for SARS-CoV-2 infection	29, 30
	Showing that SARS-CoV-2 infection can cause robust transcriptomic and morphological signatures of damage in cardiomyocytes	31
iPSC-derived neural progenitor cells	Assessing susceptibility of these stem cells to SARS-CoV-2 infection	34
iPSC-derived neural cells	Testing SARS-CoV-2 neurotropism	35
Organoids		
Lung organoids (hESC)	Identifying drug candidates that block SARS-CoV-2 entry	41
Bronchial organoids (commercially available cryopreserved human bronchial epithelial cells)	Studying SARS-CoV-2 infection and drug screening	42
Bronchial organoids (primary human bronchial epithelial cells)	Identifying potential key genes for SARS-CoV-2 infection	43
Tonsil organoids (human tonsil epithelium)	Studying SARS-CoV-2 infection	44
Intestinal organoids (human small intestinal cells)	Demonstrating that SARS-CoV-2 readily replicates in gut enterocytes	47, 48
Intestinal organoids (bat intestinal cells)	Providing evidence that SARS-CoV-2 infects bat intestinal cells	48
Intestinal organoids (human colon)	Revealing that human intestinal epithelium supports SARS-CoV-2 infection, replication, and de novo infectious virus production	49
Liver organoids (liver bile duct–derived progenitor cells)	Showing that SARS-CoV-2 infection induces cell death of cholangiocytes and bile acid accumulation	54
Liver organoids (iPSCs)	Documenting that hepatocytes and cholangiocyte organoids are permissive to SARS-CoV-2	24
Kidney organoids (human embryonic stem cells)	Revealing that human recombinant soluble ACE2 inhibits SARS-CoV-2 infection in these organoids	55
Blood vessel organoids (iPSCs)	Showing that SARS-CoV-2 infection can be reduced by human recombinant soluble ACE2	55
Brain organoids (iPSCs)	Addressing SARS-CoV-2 neurotropism Identifying that SARS-CoV-2 mainly targets neural progenitor cells and cortical neurons Evaluating therapeutic strategies	34, 35, 54, 59, 60
Brain organoids (hESCs)	Providing a model for investigating SARS-CoV-2 entry into the human brain	58
Choroid plexus organoids (iPSCs)	Showing greater SARS-CoV-2 infection of choroid plexus organoids compared to other brain regions that cause cell death and altered secretory function	35

(*Continued*)

Table 2 (*Continued*)

Model type (Origin)	Application(s)	Reference(s)
Organs-On-Chips		
Primary lung epithelium-on-a-chip	Modeling SARS-CoV-2 entry, replication, and host cytokine production Drug screening	62
Vascularized lung-on-a-chip	Studying SARS-CoV-2 replication	63
Microengineered alveolus-on-a-chip	Reconstituting key features of the human alveolar-capillary barrier in SARS-CoV-2 infection	64
Intestine-on-a-chip	Recapitulating intestinal injury and immune response induced by SARS-CoV-2	65
Tissue explants		
Human lung tissue explants	Demonstrating the viral kinetics, cell tropism, and innate immune response profile of SARS-CoV-2	68, 70
Organotypic human bronchial epithelial cells	Revealing that ciliated cells are a major target of SARS-CoV-2 infection	69, 70
Organotypic human airway epithelial cultures	Elucidating that SARS-CoV-2 causes plaque-like cytopathic effects in these cells	71
Ex vivo cultures of human conjunctiva	Showing that conjunctival epithelium is a potential portal for SARS-CoV-2 infection	70

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; RD, rhabdomyosarcoma; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine subtype 2.

SARS-CoV-2 infections (12). One such cell line used to replicate and isolate SARs-CoV-2 is VeroE6, originally isolated from kidney epithelial cells of an African green monkey (13–15). Caco-2, an epithelial cell line derived from a human colorectal adenocarcinoma, has been used to assess COVID-19 pathology. Ellinger et al. (16) recently used the Caco-2 cell line to screen 5,632 compounds for their potential to inhibit viral-induced cytotoxicity. Caco-2 cells have also revealed pathways relevant for SARS-CoV-2 infections, including nucleic acid metabolism, protein homeostasis, translation, and splicing (17). The Calu-3 cell line, originally derived from human lung adenocarcinoma, is another preclinical model of pulmonary viral infections that is used to study SARS-CoV-2 (13, 18–20).

Chu et al. (21) analyzed the differential susceptibility of 25 cell lines and compared the replication capability of SARS-CoV-2, as assessed by quantitative reverse transcription PCR (22). These cell lines were mainly derived from different human tissues, including from the respiratory tract (A549, Calu-3, and HFL), gastrointestinal tract (Caco-2), liver (Huh7), cervix (HeLa), kidney (293T), brain (U251), and muscle (RD). Moreover, several cell lines originated from nonhuman species such as bat (RLL, RLK, RSL, and RSK), dog (MDCK), pig (RK-15), rabbit (RK-13), cat (CRFK), porcupine (Pok), and NHPs (VeroE6, FRhK4, LLCMK2). The findings revealed that SARS-CoV-2 infected and replicated to comparable levels in human Caco-2 and Calu-3 cells over a period of 120 h.

This study provided valuable information and novel insights into SARS-CoV-2 cellular tropism, replication kinetics, and cell damage, but the authors noted several limitations with these cell lines. First, tropism among the cell lines might not exactly reflect how SARS-CoV-2 replicates and affects human organs in a physiological condition. Second, the authors used only one viral isolate (but which was highly homologous to other SARS-CoV-2 reported isolates). Third, human organs and tissues contain multiple cell types that express ACE2 heterogeneously.

Therefore, using immortalized or cancer cell lines might not fully reveal SARS-CoV-2 infection rates and mechanisms in different cell types. In addition, most of these human cancer cell lines carry tumor-associated mutations, such as in P53, which is known to regulate replication of SARS-CoV (23). This issue raises concerns as to whether these cancer cell lines recapitulate SARS-CoV-2 biology in a physiological context. Moreover, as these cells mainly originate from cancers, their proliferation and lack of polarization might impact the transfection efficiency of the virus (24).

2.3. iPSC-Derived Stem Cells

Although primary cell culture models have been frequently used to study SARS-CoV-2, their short in vitro maintenance is a major limitation, and because of genomic alterations, the cell lines may not faithfully respond to SARS-CoV-2 infections. By contrast, iPSCs offer the potential to study cells more reminiscent of human tissue. The transcriptome of iPSC-derived cells is similar to that of their respective primary counterparts and allows them to respond to immune stimuli. To date, several iPSC-derived cell types have provided a platform for drug development, systematic exploration of SARS-CoV-2 viral tropism, and studying cellular responses to infection.

Surendran et al. (25) generated lung epithelial cells from human iPSCs using a three-step protocol. Another research group presented an in vitro human model that could simulate the apical infection of distal lung epithelium with SARS-CoV-2 by developing iPSC-derived alveolar epithelial type 2 cells (iAT2s) as facultative progenitors of this tissue; SARS-CoV-2 infection was able to induce rapid global transcriptomic alterations in these cells (26). In addition, Abo et al. (27) compared iPSC-derived iAT2s and iPSC-derived airway epithelial cells with primary lung epithelial cell controls, concentrating on the expression levels of genes related to COVID-19 modeling. The results indicated that these two generated cells express key transcripts associated with lung identity, e.g., ACE2 and TMPRSS2. Duan et al. (28) used directed differentiation of human induced pluripotent stem cells (hiPSCs) to establish a lung/macrophage coculture system to simulate host-pathogen interaction and immune response caused by SARS-CoV-2 infection. This strategy overcomes a main concern about histocompatibility when studying human immune cells with other cell types and sustains unlimited cell resources for reliably modeling the immunology of macrophages and human lungs during infection. Using hiPSC-derived cardiomyocytes (hiPSC-CMs), Bojkova et al. (29) demonstrated that these cells are permissive for SARS-CoV-2 infection, as detected by intracellular double-stranded viral RNA and viral S protein expression. In addition to confirming the susceptibility of hiPSC-CMs to SARS-CoV-2 infection (30), it was shown that this infection can cause transcriptomic and morphological signatures of damage in these cells (31).

Accumulating evidence suggests that the human brain is another extrapulmonary target of this virus. Of note, 36% of SARS-CoV-2-infected patients displayed neurological conditions (32), and the virus has been detected in postmortem brain tissue of COVID-19 patients (33). Zhang et al. (34) reported SARS-CoV-2 infection of iPSC-derived human neural progenitor cells, while Jacob et al. (35) investigated susceptibility of iPSC-derived neurons, astrocytes, and microglia in monolayer cultures. They observed modest numbers of infected neurons and astrocytes and a high level of infection in choroid plexus epithelial cells.

2.4. Organoids

The emergence of human in vitro three-dimensional (3D) cell culture approaches using stem cells has received widespread attention due to their potential to overcome the limitations of classical two-dimensionally cultured and immortalized cell lines (36). Organoids are 3D structures mainly established from pluripotent stem cells (PSCs) or, alternatively, from multipotent adult stem cells resident in the tissue. They are composed of tissue-specific cell types that self-organize through

cell sorting and spatially restricted lineage commitment to generate cell assemblies with functional and architectural features of the corresponding tissue. Organoids optimally contain a full range of the differentiated cell types present in the target organ (37).

Numerous organoids have been employed to study SARS-CoV-2 infection at a level between single cells and tissue (38). As the primary SARS-CoV-2 infection target is the respiratory tract, several research groups have used lung organoids to mimic the human lung (39, 40). Han et al. (41) developed a lung organoid model using human PSCs and screened several US Food and Drug Administration (FDA)-approved drug candidates such as mycophenolic acid and imatinib as inhibitors of SARS-CoV-2 entry. Suzuki et al. (42) established human bronchial organoids (hBOs) from commercially available cryopreserved human bronchial epithelial cells. This model contained basal, club, ciliated, and goblet cell types, and treatment of SARS-CoV-2-infected hBOs with the TMPRSS2 inhibitor camostat significantly reduced viral copies in this model. Cytokine/cytokine receptor interactions, apoptosis, and P53 signaling are the main cellular mechanisms activated in these organoids following SARS-CoV-2 infections (43). Since palatine tonsils are in the front line of the immune system's defense against pathogens, tonsil epithelial cell–derived organoids reflect the distinctive characteristics of the corresponding tissue and have been proposed as a platform for studying coronavirus infections and COVID-19 biology (44).

Respiratory symptoms dominate clinical aspects of COVID-19, but involvement of the gastrointestinal system can occur as well (45, 46). A study with human small intestinal organoids suggested infection and replication of SARS-CoV-2 in human intestinal epithelium (47). Differentiated enterocytes in these organoids were readily infected by SARS-CoV-2, as demonstrated by confocal and electron microscopy. Zhou et al. (48) used expandable intestinal organoids derived from horseshoe bats of the species *Rhinolophus sinicus* that could recapitulate the bat intestinal epithelium. Due to their susceptibility to SARS-CoV-2 infections, these organoids sustain robust viral replication. The same group also confirmed SARS-CoV-2 replication in human intestinal organoids as well as upregulation of type III interferon responses. This notion was further supported by Stanifer et al. (49), who showed that SARS-CoV-2 could infect the human gastrointestinal tract and efficiently produce de novo viruses. Interestingly, they found that the type III interferon, which is naturally made by cells in response to viral infection, can be used as an antiviral strategy to protect infected cells.

The liver abundantly expresses ACE2, and liver damage can occur with SARS-CoV-2 infections (50–52). The average expression level of ACE2 in hepatocytes is 20-fold less than in cholangiocytes. This level of expression in cholangiocytes is comparable to that of lung AT2 cells (53). Zhao et al. (54) employed a previously established model of liver ductal organoids to demonstrate that these organoids are susceptible to SARS-CoV-2. RNA sequencing revealed a set of 337 differentially expressed genes in the infected organoids. The data indicate that SARS-CoV-2 infection induces cell death of cholangiocytes that can subsequently cause bile acid accumulation. This issue was further investigated by Yang et al. (24), who evaluated SARS-CoV-2 tropism in various human cells and organoids and found that human hepatocyte and cholangiocyte organoids are permissive to SARS-CoV-2 infection. Human blood vessel and kidney organoids have also been isolated, and human recombinant soluble ACE2 significantly reduced viral growth in these infected organoids (55).

Many hospitalized COVID-19 patients exhibit neurological manifestations ranging from loss of smell and headache to confusion and disabling strokes (56). Brain organoids can address several questions concerning the neurotropism and mechanisms of neuropathogenesis (57–59).

Ramani et al. (60) provided an initial insight into the unexpected neurodegenerative-like effects of SARS-CoV-2. The authors found that the Düsseldorf isolate of SARS-CoV-2 preferably targets neurons in 3D human brain organoids, leading to altered Tau distribution and phosphorylation

and eventually causing neuronal death. This finding was further supported by experiments that showed SARS-CoV-2 infection in 3D human brain organoids was localized to NESTIN-positive and TUJ1-expressing cells, suggesting that SARS-CoV-2 can directly target neural precursor cells and cortical neurons (34). Jacob et al. (35) investigated the susceptibility of region-specific brain organoids to SARS-CoV-2 and found greater infection of choroid plexus organoids that caused cell death and transcriptional dysregulation related to increased inflammation and altered secretory function. In spite of its many advantages, an important limitation of organoid technology is the lack of communication between organs as occurs in vivo. Thus, the findings require validation in more complex models. Human organoid culture remains under development, and numerous efforts to advance this technology are still in progress.

2.5. Organ-on-a-Chip

Organ-on-a-chip technology offers a special platform to mimic human organ infection. Human organ chips are engineered microfluidic devices populated with human cells that recapitulate tissue-tissue interfaces, fluid flows, mechanical cues, and organ-level physiology (61). Ingber and colleagues (62) developed organ chip microfluidic culture devices lined by primary human lung airway epithelium. The cells are cultured under ALI conditions and fed by continuous flow of medium. This model mimics virus entry, replication, and host cytokine production and represents a particularly useful platform to expedite drug repurposing. Recently, a vascularized lung-on-a-chip infection model was developed that consists of a coculture of primary human alveolar and human lung microvascular endothelial cells with the optional addition of CD14-positive macrophages. This model was used to study SARS-CoV-2 replication and host response to infection (63). The use of an alveolus chip was reported by Zhang et al. (64), who were able to reconstitute the key features of the alveolar-capillary barrier. Notably, they showed distinct responses of two cell types to SARS-CoV-2 infection, including activation of the type I interferon signaling cascade in epithelium and the Janus kinase–signal transducer and activator of transcription (JAK-STAT) signaling pathway in the endothelium. Guo et al. (65) created a microengineered intestine-on-a-chip device to mirror the pathophysiological features and immune responses in COVID-19. This model contains cocultured human intestinal epithelial (Caco-2) cells and mucin-secreting HT-29 cells lined in the upper channel and human umbilical vein endothelial cells in the lower channel under fluidic conditions. Collectively, organ-on-a-chip-based assays provide a platform to study SARS-CoV-2-induced pathology in real time and at high resolution in a human-relevant environment.

2.6. Tissue Explants

The study of human cell-cell and cell-pathogen interactions that occur in the context of tissue cytoarchitecture is important for deciphering the underlying mechanisms of many normal and pathogenic processes (66). In this regard, tissue explants or organotypic cultures are robust in vitro models that are established from surgical resection material and can be highly suitable platforms at a level between dissociated cell cultures and animal models. These models offer several unique advantages compared to other in vitro approaches: They generally retain the tissue architecture of their originated region, preserve cell-cell and cell-matrix interaction, and allow direct real-time observation (67). With respect to SARS-CoV-2 infection, human lung tissue explant is one of the most useful ex vivo models to compare the viral kinetics, cell tropism, and innate immune response profile of SARS-CoV-2 and SARS-CoV (68). This ex vivo culture showed more efficient replication but less host interferon and proinflammatory response following SARS-CoV-2

infection compared to primary cell culture. Also, single-cell RNA sequencing and electron microscopy on organotypic human bronchial epithelial cells infected with SARS-CoV-2 revealed that ciliated cells are a major target of infection (69).

Besides lung explants, ex vivo cultures of human bronchus and conjunctiva have been employed to study SARS-CoV-2 infection. These models have demonstrated that the virus can infect type 1 pneumocytes in the lung; in ciliated, mucus-secreting, and club cells of bronchial epithelium; and in conjunctival mucosa (70). In addition, Zhu et al. (71) recently characterized the replication dynamics, cell tropism, and morphogenesis of SARS-CoV-2 in organotypic human airway epithelial cultures. The results showed that SARS-CoV-2 causes plaque-like cytopathic effects in these cells. Apoptosis, cell fusion, destruction of epithelium integrity, and cilia shrinking and beaded changes were observed in the plaque-like regions. Thus, tissue explants and organotypic cultures can be useful platforms to enhance insight into SARS-CoV-2 infection.

3. ANIMAL MODELS FOR SARS-COV-2 RESEARCH

In vitro approaches may not provide insight into the harmful in vivo effects. There is thus an urgent need to develop animal models that are amenable to investigating effects of SARS-CoV-2 on target organs and systemic effects of the infection. A range of animal models have been developed and used (72–75) (**Table 3**). Each model has benefits and limitations (**Table 4**).

3.1. Mouse

Since mouse angiotensin-converting enzyme 2 (mACE2) does not support SARS-CoV-2 binding as does hACE2, laboratory strains of mice are not readily infected by SARS-CoV-2 (76, 77). Thus, multiple interventions based on genetic modifications have been introduced to facilitate SARS-CoV-2 susceptibility in order to provide more physiologically relevant platforms for modeling the infection. In this regard, Sun et al. (78) described an adenoviral transduction-based mouse model (Ad5-hACE2-sensitized mice) that can be infected with SARS-CoV-2. These mice can develop pneumonia, characterized by weight loss, severe pulmonary pathology, and high-titer virus replication in the lungs. Thus, this model provides a tool to understand host factors involved in viral infection and clearance as well as potential therapeutic modalities. Although Bao et al. (79) recently reproposed their potentially useful hACE-2 transgenic mice for SARS-CoV-2 studies, the viral replication in these mice was suboptimal, with pathological changes in the lung and weight loss being minimal. Another suggested model is cytokeratin-18 (K18)-hACE2 transgenic mice in which hACE2 expression is driven by the epithelial cell K18 promoter. These transgenic mice shares many features of severe COVID-19 infection (80). Jiang and colleagues (2020) introduced a HFH4-hACE2 transgenic mice that could be infected with SARS-CoV-2. This model was able to recapitulate a number of the symptoms and pathology seen in COVID-19 patients and pre-exposure to SARS-CoV-2 protected these mice from severe pneumonia (81). A mouse model generated using clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated 9 knock-in technology to express hACE2 was predicted to be a useful platform to study SARS-CoV-2 infection and transmission, but fatalities were not observed in this model (82). Hassan et al. (83) transiently transduced a replication-defective adenovirus encoding hACE2 into the lungs of commercially available mice, which are permissive for SARS-CoV-2. This model is widely available for high-throughput drug and vaccine testing. Pujhari & Rasgon (84) developed two new strategies for generating mice with humanized lungs and immune systems as a model to define the mechanisms of SARS-CoV-2 infection. The first focused on humanized mice with ectopically grafted human lung (85), while the second concentrated on the generation of functional lungs via conditional blastocyst complementation using PSCs (86).

Table 3 In vivo models used in COVID-19 research

Type	Key Points	Reference(s)
Ad5-hACE2-sensitized mice	hACE2 was provided by adenovirus transduction Mouse model that can be infected with SARS-CoV-2; can aid in understanding host factors involved in viral infection/clearance and potential therapeutics	78
hACE2 transgenic mice	Mice generated by microinjection of the mouse Ace2 promoter driving the human ACE2–coding sequence into the pronuclei of fertilized ova from ICR mice Model used to study SARS-CoV-2 pathogenicity	79
K18-hACE2 transgenic mice	hACE2 expression driven by epithelial cell K18 promoter Introduced as a model to define the basis of lung disease in SARS-CoV-2 infection	80
HFH4-hACE2 transgenic mice	Mice express human ACE2 under the control of a lung ciliated epithelial cell–specific HFH4/FOXJ1 promoter Model used to recapitulate a number of infection symptoms and pathology in COVID-19 patients	81
hACE2 mice	Humanized ACE2 mouse by CRISPR-Cas9 knock-in technology A tool to study SARS-CoV-2 transmission and pathogenesis and to evaluate vaccines and therapeutics	82
hACE2 transduced mice	Mice transduced with adenoviruses encoding human ACE2 Model is now widely available for high-throughput drug and vaccine testing	83
Wild-type BALB/c mice	Uses mouse-adapted SARS-CoV-2 strain at passage 6 (called MASCp6) Model used to validate protective efficacy of candidate vaccines	87
Mouse-adapted SARS-CoV-2 (SARS-CoV-2 MA)	Uses recombinant virus that utilizes mACE2 for entry Model used to demonstrate age-related disease pathogenesis; supports the clinical use of pegylated IFN-λ1a as a treatment for human COVID-19	88
Syrian hamster	Infection of hamsters revealed that a SARS-CoV-2 deleted variant (Del-mut-1) has attenuated ability to cause disease in this model	89
	Surgical mask partition in hamster model can reduce SARS-CoV-2 transmission by respiratory droplets or airborne droplet nuclei	90
	Syrian hamster is a model that simulates the clinical and pathological manifestations of COVID-19; SARS-CoV-2 isolates efficiently replicate in their lungs and cause severe pathological lesions The passive transfer of convalescent serum to naïve hamsters inhibited virus replication in their lungs	92, 93
	SARS-CoV-2 is efficiently transmitted from inoculated hamsters to naive hamsters by direct contact and via aerosols	94
	SARS-CoV-2-infected hamsters can develop neutralizing antibodies, which protect them from reinfection	92–95
	SARS-CoV-2 replication in the upper and lower respiratory tract was independent of the age of the hamster Rapid lung recovery was observed only in young hamsters	97
	Following SARS-CoV-2 infection, the STAT-dependent interferon responses play a critical role in the pathogenesis and control of the virus	98
Ferrets	Ferrets are highly susceptible to SARS-CoV-2 infection	102, 103
	Ferrets effectively transmit the virus by direct or indirect contact, recapitulating human transmission	104, 105
	The antiviral efficacies of several FDA-approved drugs against SARS-CoV-2 have been assessed in infected ferrets	106
	The alteration of gene expression from short-term to long-term SARS-CoV-2 infection was evaluated in infected ferrets	107

(*Continued*)

Table 3 (*Continued*)

Type	Key Points	Reference(s)
Nonhuman primates, general	Apes, African and Asian monkeys, and some lemurs are likely highly susceptible to SARS-CoV-2	110
Rhesus macaque	Exhibits high ACE2-spike activity and is susceptible to SARS-CoV-2 infection	111–113
	Early remdesivir treatment in SARS-CoV-2 infection had a clinical benefit	114
	SARS-CoV-2 infection induced protective immunity against subsequent reinfection	115, 116
	DNA vaccine encoding full-length S protein protected them from SARS-CoV-2 infection	117
	ChAdOx1 nCoV-19 vaccine prevented SARS-CoV-2 pneumonia	118
	Inactivated SARS-CoV-2 virus vaccine (PiCoVacc) induced SARS-CoV-2-specific neutralizing antibodies	119
	Single dose of Ad26 vector-based vaccine protected against SARS-CoV-2 infection	120
African green monkey	High level of SARS-CoV-2 replication and pronounced respiratory tract infection	121–123
Cynomolgus macaques	Useful model to study SARS-CoV-2 infection	124, 126
	NVX-CoV2373 vaccine protected against SARS-CoV-2 infection	127
Crab-eating macaques	Susceptible to SARS-CoV-2 infection	128

Abbreviations: ACE2, angiotensin-converting enzyme 2; Ad26, adenovirus serotype 26; COVID-19, coronavirus disease 2019; CRISPR-Cas9, clustered regularly interspaced short palindromic repeat–CRISPR-associated 9; FDA, US Food and Drug Administration; ICR, Institute for Cancer Research; IFN-λ1a, interferon λ1a; K18, cytokeratin-18; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 4 Benefits and limitations of in vivo models in COVID-19 research

Experimental model	Benefits	Limitations
Mouse	Small size, ease of use, and rapid breeding make mice well suited for rapid screening of antiviral drugs and vaccines Enable exploration of effects of genetic diversity and background	Mismatched ACE2 receptors Even genetically engineered mice are not a perfect substitute for human infection
Hamster	Naturally susceptible to SARS-CoV-2 infection Infected golden Syrian hamsters closely mimic the mild to moderate disease of human patients Can be used to study viral transmission Suitable for evaluation of antiviral agents and candidate vaccines	May not reflect human pharmacokinetics May not metabolize prodrugs as do humans
Ferret	Lung and airway physiology are similar to those of humans Useful model in the study of disease transmission	May not reflect human pharmacokinetics Low viral titer in lungs
Nonhuman primate	The closest model to human infection, as their innate and adaptive immunity, physiology, and anatomy are similar to those of humans Excellent model to assess by repeated sampling and imaging, given their size and longevity Similar pharmacokinetics to humans	High costs and ethical considerations Small sample sizes

Abbreviations: ACE2, angiotensin-converting enzyme 2; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Besides genetically modified mice, COVID-19 pathology has been studied in wild-type mice infected with adapted SARS-CoV-2 variants. Gu et al. (87), through inoculation with mouse-adapted SARS-CoV-2, efficiently infected young and aged BALB/c mice, resulting in moderate pneumonia and inflammatory responses. Dinnon et al. (88) circumvented the inefficient interaction of viral S protein and mACE2 using reverse genetics to remodel their binding interface, resulting in a recombinant virus (SARS-CoV-2 MA) that utilizes mACE2 for entry. This SARS-CoV-2 MA replicated in both upper and lower airways of mice irrespective of their ages. Although both wild-type mouse models were suggested as reliable platforms for drug and vaccine screening, they may not fully recapitulate all aspects of the human disease (72).

3.2. Syrian Hamster

Syrian golden hamster is another small rodent that has been widely used to model SARS-CoV-2 infection (89, 90), although no mortality was observed after virus infection (91). Hamster ACE2 has high-affinity binding of the SARS-CoV-2 S glycoprotein receptor–binding domain. Chan et al. (92) showed that hamster ACE2 affinity to the S protein is the highest observed apart from humans and rhesus macaques. Furthermore, SARS-CoV-2 isolates efficiently replicate in hamster lungs, causing severe pathological lung lesions following intranasal infection (93). Notably, hamsters infected with SARS-CoV-2, besides showing clinical signs of disease, can transmit the virus to naïve cohoused animals by direct contact and/or via aerosols (94). Although this model can recapitulate certain aspects of human pathogenesis, infected animals can return to their original weight within 2 weeks postinfection and develop neutralizing antibodies that protect them against subsequent rechallenge with SARS-CoV-2 (95, 96). In an elegant experiment, Osterrieder et al. (97) compared the course of infection in young and aged hamsters. The authors found an identical virus replication rate in the upper and lower respiratory tract of both groups, but rapid lung recovery was only observed in young hamsters, a result of relevance to the age-dependent differences in humans. Boudewijns et al. (98), using a unique STAT2-knockout hamster, revealed a dual role of this signaling pathway in driving severe lung injury and also restricting systemic virus dissemination. This finding underlines the importance of STAT2-dependent interferon responses in the pathogenesis and control of the virus during infection and may identify new strategies for COVID-19 treatment. Suresh et al. (99) assessed the tissue-specific expression pattern of ACE2 in different organs of hamster and found that kidney, small intestine, esophagus, tongue, brain, and liver express this receptor but lung, tracheal epithelial cells, and large intestine lacked ACE2 expression. Overall, the hamster model is a useful platform to study transmission, pathogenesis, and treatment of and vaccination against SARS-CoV-2.

3.3. Ferret

Ferrets have been useful in reproducing human diseases caused by influenza virus and SARS-CoV, as their respiratory tract is anatomically comparable to humans and they express ACE2 abundantly on type II alveolar and granular epithelial cells in the trachea and bronchi (100, 101). In addition, ferret ACE2 contains critical SARS-CoV binding residues that render them susceptible to SARS-CoV-2 (102, 103). Infected ferrets show high virus titers in the upper respiratory tracts, and consequently, highly efficient ferret-to-ferret transmission can occur via direct or indirect contact (104). The ferret transmission pattern has been investigated by Richard et al. (105) who detected infected viruses in indirect recipients, suggesting that the SARS-CoV-2 ferret model recapitulates human-to-human transmission. Based on its susceptibility, the ferret model has been used to assess a number of FDA-approved drugs, which have been suggested as antiviral candidates against

SARS-CoV-2 and yielded results that may help facilitate the selection of antiviral treatments for COVID-19 (106). In addition, alteration of gene expression in short- and long-term-infected ferrets has been shown using bioinformatics approaches: During the course of infection, the stemness, development, and Wnt- and cytoskeleton remodeling–related pathways mainly change in the early phase of lung infection, while the ECM, immune response, G protein–coupled receptor, and cell adhesion–related pathways are affected in the later phase (107). Given the rapid spreading of SARS-CoV-2 in humans, ferrets can be a useful model for understanding COVID-19 transmission dynamics.

3.4. Nonhuman Primates

NHP models of SARS-CoV-2 are the gold standard for modeling human pathogenesis and for testing clinical interventions due to their genetic homology and anatomic similarity to humans (108). Based on the fidelity of NHP models for the investigation of the 2003 SARS-CoV outbreak (109), these models were reconsidered to assess SARS-CoV-2 infection. A range of NHP models have been used to study COVID-19 with varying susceptibility, which likely depends on species-specific properties of ACE2 (110). Owing to their high ACE2-spike activity, rhesus macaques are a NHP susceptible to SARS-CoV-2 infection (111–113) and have been extensively used to assess the efficacy of medical countermeasures. The efficacy of remdesivir (GS-5734), a nucleotide analog prodrug with broad antiviral activity, was investigated in a rhesus macaque model of SARS-CoV-2 (114). Remdesivir-treated animals did not show signs of respiratory disease, and pulmonary infiltration and virus load were reduced following a first dose. This study concluded that the early initiation of treatment with remdesivir can benefit infected animals. Rechallenge of infected rhesus macaques with SARS-CoV-2 resulted in a viral load reduction that suggests primary infection–induced protective immunity against reinfection in these models (115, 116).

Numerous vaccine candidates are currently being developed, and their efficacy is evaluated in NHP models. Yu et al. (117) used rhesus macaques to test a series of DNA vaccines expressing different forms of the SARS-CoV-2 S protein. The vaccine encoding the full-length S protein resulted in a dramatic reduction of viral replication in both the upper and lower respiratory tract after challenge with SARS-CoV-2. Doremalen et al. (118) found that vaccination of rhesus macaques with ChAdOx1 nCoV-19 induced balanced Th1/Th2 humoral and cellular immune responses, which prevent SARS-CoV-2 infection. Another vaccine developed by Gao et al. (119) is an inactivated SARS-CoV-2 virus vaccine (PiCoVacc), which induces the synthesis of SARS-CoV-2-specific neutralizing antibodies in macaques. In addition, the immunogenicity and protective efficacy of a single dose of adenovirus serotype 26 (Ad26) vector–based vaccines expressing the SARS-CoV-2 S protein were assessed in macaques. The single-shot Ad26 vaccine protected these animals against SARS-CoV-2 infection, and it is currently being evaluated in clinical trials (120).

African green monkeys are another NHP model for COVID-19 that replicates most facets of the human disease. These monkeys support high levels of SARS-CoV-2 replication and are susceptible to pronounced respiratory tract infection (121, 122). Importantly, subclinical infection of African green monkeys with SARS-CoV-2 resulted in prolonged shedding of infectious virus from both respiratory and gastrointestinal tracts (123).

A comparison of how human coronaviruses, including SARS-CoV, MERS-CoV, and SARS-CoV-2, develop in cynomolgus macaques showed that this NHP could be a predictive model to test preventive and therapeutic strategies against COVID-19 (124, 125). Salguero et al. (126) provided evidence that, though rhesus monkeys are considered to be the optimal model species, SARS-CoV-2 infection progresses similarly in both species. Guebre-Xabier et al. (127) revealed the efficacy of a SARS-CoV-2 subunit vaccine (NVX-CoV2373) in cynomolgus macaques. This

vaccine protected the animals against SARS-CoV-2 replication in both upper and lower respiratory tracts. In crab-eating macaques (*Macaca fascicularis*), serial computed tomography demonstrated that bilateral intrabronchial instillation of SARS-CoV-2 led to mild-to-moderate lung abnormalities (128).

Lu et al. (129) compared three species of NHPs, including rhesus macaques (*Macaca macaque*), crab-eating macaques (*Macaca fasicularis*), and common marmosets (*Callithrix jacchus*), that were challenged with the same strain of SARS-COV-2 to identify the most suitable model for COVID-19. Based on analysis of clinical symptoms, viral replication, tissue tropism, and host responses to SARS-CoV-2 infection among the three species from two families of NHPs, rhesus macaques presented the strongest response to SARS-CoV-2. Hence, this animal closely recapitulated human-like conditions. Overall, NHPs are excellent animal models to better understand aspects related to human pathology in the current COVID-19 pandemic.

4. COMPUTATIONAL AND MATHEMATICAL MODELS

Apart from the above-described in vivo and in vitro models, several computational and mathematical models are being used to study SARS-CoV-2 (130, 131). These computational models have been widely employed to screen potential drugs against SARS-CoV-2 (132, 133) and provide new components and targets for further in vitro and in vivo investigation of SARS-CoV-2 (134). Also, numerous computational and mathematical models predict the trend of COVID-19 transmission and number of infected individuals so as to help epidemic prevention and control measures (135–138).

5. THERAPEUTIC APPROACHES FOR THE COVID-19 PANDEMIC

The global pandemic has created an urgent need to identify and validate therapeutic agents that can rapidly become part of clinical care. Antiviral drugs and host immunomodulators are the main approaches that may support recovery from COVID-19 (139). Antiviral agents can impair viral replication either by directly acting on viral targets or by targeting host proteins required for viral replication. Direct-acting antiviral compounds target SARS-CoV-2 proteins, including the S protein, proteases, helicase, and RNA-dependent RNA polymerase (RdRp) (140). Remdesivir (GS-5734), an inhibitor of RdRp with in vitro and in vivo inhibitory activity against SARS-CoV-2 (**Table 5**), was the first antiviral drug that received FDA approval in October 2020 for the treatment of COVID-19. This drug shortened the time to recovery in adults who were hospitalized with COVID-19 and showed evidence of lower respiratory tract infection (141). Later, in November 2020, WHO issued a conditional recommendation against the use of remdesivir in hospitalized patients, based on the lack of evidence that remdesivir improves survival and other outcomes in these patients (142).

Host-encoded proteins such as kinases and proteases employed by SARS-CoV-2 are potential targets of antiviral agents (143). Immunomodulatory agents, e.g., interleukin (IL)-1 (144) and IL-6 (145) receptor antagonists, might be beneficial for COVID-19 patients by attenuating an excessive host immune response. Based on evidence from several clinical trials (146), in September 2020, WHO issued a guideline on the use of dexamethasone and other corticosteroids (which have anti-inflammatory and immunosuppressant effects) as effective treatments for COVID-19. WHO recommends that corticosteroids (i.e., dexamethasone, hydrocortisone, or prednisone) be given orally or intravenously for the treatment of patients with severe and critical COVID-19.

The synthesis and investigation of potential new drugs is a time-consuming process that is not well suited for the COVID-19 pandemic. Accordingly, off-label use (repurposing) of existing

drugs is a promising approach, as it reduces development timelines and costs. Due to a lack of well-vetted guidelines, initial clinical use of such drugs in the COVID-19 pandemic has been uncoordinated. Some drugs were tested without a strong rationale or evidence to support their use against SARS-CoV-2. There remains a high demand to accelerate identification of agents with efficacy and safety for treating COVID-19. To this aim, both in vitro and in vivo experimental models of SARS-CoV-2 can elucidate aspects of pharmacology, toxicology, and immunology of the therapeutic strategies, which may evolve as understanding of COVID-19 biology improves (147). **Table 5** lists some repurposed antiviral drugs that have been tested in experimental models. It is clear that showing positive effects in a single nonhuman cell line assay cannot confirm the

Table 5 Compounds, their mode of action, and treatment outcome in models of SARS-CoV-2

Mechanism of action	Drug	Experimental model	Outcome	Reference(s)
Blockade of viral entry	Nafamostat	Vero E6	Inhibited SARS-CoV-2 infection	149
		Vero E6/TMPRSS2	Inhibited SARS-CoV-2 S protein–mediated fusion and SARS-CoV-2 infection in vitro in a cell type–dependent manner	150, 151
		Calu-3		
	Umifenovir (Arbidol)	Vero E6	Inhibited SARS-CoV-2 in vitro	152–154
Viral protease inhibitor	Lopinavir/ ritonavir	Vero E6	Inhibited SARS-CoV-2 in vitro	152, 155–157
		Ferret	Marginally reduced overall clinical scores of infected ferrets but did not affect in vivo virus titers	106
Viral RNA polymerase inhibitor	Favipiravir	Vero E6	Not effective against SARS-CoV-2 infection in vitro	149, 155
			Rapid incorporation by the viral RNA polymerase complex resulted in SARS-CoV-2 lethal mutagenesis	158
		Syrian hamster	At high doses, antiviral activity in SARS-CoV-2-infected hamsters	159
	Remdesivir	Vero E6	Highly effective in the control of COVID-19 infection in vitro	149, 152, 155, 160
		Huh-7	Inhibited virus infection in human liver cancer cells	149
		Calu-3	Inhibited SARS-CoV-2 replication with submicromolar EC_{50}	160
		Primary HAE	Inhibited SARS-CoV-2 replication	160
		Chimeric SARS-CoV encoding SARS-CoV-2 RNA polymerase in mice	Diminished lung viral load and improved pulmonary function compared to vehicle-treated animals	160
		Ad5-hACE2 transduced mice	Treatment 1 day prior to infection with continued dosing twice daily decreased weight loss, accelerated viral clearance, and diminished cellular infiltration in the lungs	78
		Rhesus macaques	Treatment initiated early during infection had clinical benefit in animals infected with SARS-CoV-2	114

(*Continued*)

Table 5 (*Continued*)

Mechanism of action	Drug	Experimental model	Outcome	Reference(s)
Miscellaneous mechanisms	Berberine	Vero E6	EC_{50} in this model of SARS-CoV-2 infection was 10.6 μM	152
	Chloroquine	Vero E6	Inhibited COVID-19	149, 152
		Calu-3	Did not inhibit infection of human lung cells with SARS-CoV-2	19
	Hydroxy-chloroquine	Vero	More potent than chloroquine in inhibiting SARS-CoV-2	161
		Vero E6	Inhibited SARS-CoV-2 infection	162
			Synergy with azithromycin on SARS-CoV-2	163
			Displayed antiviral activity	164
		Primary HAE	Lacked antiviral activity	164
		Ferret	Marginally reduced overall clinical scores but did not significantly affect in vivo viral titers	106
		Cynomolgus macaques	Data in this model do not support its use as an antiviral agent for the treatment of COVID-19 in humans	164
	Emetine	Vero E6	At 0.195 μM in combination with remdesivir (6.25 μM), there was 65% inhibition in viral load	155
			Off-target effects on acidophilic organelles (autophagosomes, lysosomes, endosomes) likely blunted SARS-CoV-2 replication	165

Abbreviations: COVID-19, coronavirus disease 2019; EC_{50}, 50% maximal effective concentration; hACE2, human angiotensin-converting enzyme 2; HAE, human airway epithelial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane protease serine subtype 2.

efficacy of such drugs against COVID-19. A strong rationale and evidence (ideally in randomized controlled trials) are required for their use in COVID-19 patients.

Although several therapeutic approaches have been explored for COVID-19 treatment, to mitigate the influence of the virus on public health, society, and the economy, a vaccine is urgently needed to end the global COVID-19 pandemic. Currently, more than 200 vaccines are at various stages of development (148). These vaccine candidates comprise various platforms, including protein subunit, non-replicating or replicating viral vectors, DNA, inactivated virus, RNA, virus-like particle, and live attenuated virus. Many have entered clinical trials, and more than 10 vaccines are currently in Phase 3 or 2/3 clinical trials (**Table 6**). The FDA issued an emergency use authorization for the Pfizer-BioNTech and Moderna COVID-19 vaccines, and other effective and safe vaccines will likely be available soon.

6. CONCLUSION

This review summarizes models that are currently being used to study SARS-CoV-2 infection. We describe three main categories of in vitro, in vivo, and computational/mathematical models that each provide insights into different aspects of COVID-19. The in vitro models offer platforms to elucidate the viral life cycle, delineate disease pathology in tissue explants, and recapitulate host-virus interactions. Animal models include genetically modified mouse, Syrian hamster, ferret, and NHPs, with varying susceptibility to SARS-CoV-2 infection, which is likely largely dependent on species-specific differences in ACE2. These animal models are useful to investigate the effects of

Table 6 Several vaccines that have been developed and used for COVID-19 treatment

Vaccine	Vaccine Type	Developer	Animal model(s) tested	Clinical stage[a]	Reference(s)
BNT162	mRNA via lipid nanoparticles (lipid nanoparticle–formulated, nucleoside-modified mRNA vaccine that encodes the trimerized receptor-binding domain of the spike glycoprotein of SARS-CoV-2)	Pfizer/ BioNTech	Mice Rhesus macaques	Phase 2/3	166, 167, 195
mRNA-1273	mRNA via lipid nanoparticles (encoding the prefusion-stabilized spike protein of SARS-CoV-2)	Moderna	Rhesus macaques	Phase 3	168–170
PiCoVacc	Inactivated SARS-CoV2 with aluminum hydroxide	Sinovac	BALB/c mice Wistar rats Rhesus macaques	Phase 3	119, 171
NVX-CoV2373	Full-length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	Cynomolgus macaques	Phase 3	127, 172
ChAdOx1 nCoV-19	Nonreplicating adenovirus (adenovirus vector–based vaccine, which encodes the spike protein of SARS-CoV-2)	AstraZeneca/ University of Oxford	Rhesus macaques	Phase 3	118, 173–175
Ad26COVS1	Nonreplicating adenovirus	Janssen Pharmaceuticals	BALB/c mice Hamsters Rhesus macaques	Phase 3	120, 176, 177
Adenovirus type 5 vector (Ad5CoV)	Nonreplicating adenovirus	CanSino Biological Inc./Beijing Institute of Biotechnology	NA	Phase 3	178, 179
rAd26-S + rAd5-S	Nonreplicating adenovirus	Gamaleya Research Institute	Nonhuman primates	Phase 3	180
BBV152 (Covaxin)	Inactivated SARS-CoV2	Bharat Biotech International	Mice Rats Rabbits Hamsters Rhesus macaques	Phase 3	196–199
Inactivated vaccine	Inactivated SARS-CoV-2	Sinopharm/ Wuhan Institute of Biological Products	Rhesus macaques	Phase 3	181
Inactivated vaccine (BBIBP-CorV)	Inactivated SARS-CoV-2	Sinopharm/ Beijing Institute of Biological Products	Mice Rats Guinea pigs Rabbits Cynomolgus macaques Rhesus macaques	Phase 3	182, 183

[a]According to WHO Landscape as of December 22, 2020 (148).
Abbreviations: COVID-19, coronavirus disease 2019; mRNA, messenger RNA; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

a SARS-CoV-2 infection on specific target organs and to assess systemic effects of the infection. Computational and mathematical models are employed to predict COVID-19 transmission and to screen therapeutic strategies. By combining the outcomes of the in vitro, in vivo, and computational/mathematical models, one can identify fundamental aspects of COVID-19 in humans and

have useful platforms to test therapeutic agents and vaccines for safety and efficacy. These models can help elucidate aspects of pharmacology, toxicology, and immunology of repurposed antiviral drugs and aid in optimizing drug testing during the pandemic. No single animal model can fully recapitulate human pathogenesis and predict interventional responses in humans, but the models play a key role in assessing vaccines and therapeutics.

Even with the current clinical trials for vaccines, more work is urgently needed to understand host-infection interactions; to explain the extreme severity of the effects of infection, including perhaps the role of the nervous system in a neuroinflammatory response; and to develop effective treatments for those unable to be vaccinated or for whom vaccination only provides partial or no protection.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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