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Review

Emerging Landscape of Preclinical Models for Studying COVID-19 Neurologic Diseases

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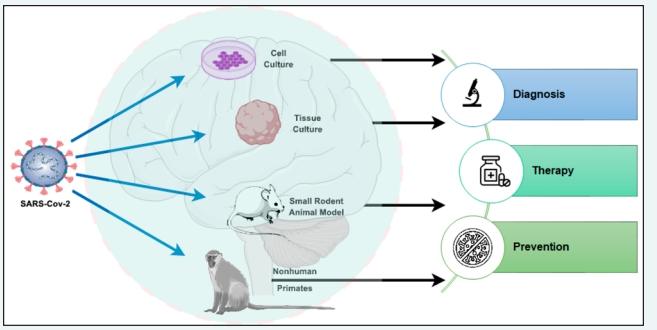
ABSTRACT: COVID-19 (Coronavirus Disease 2019) is an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) and has globally infected 768 million people and caused over 6 million deaths. COVID-19 primarily affects the respiratory system but increasing reports of neurologic symptoms associated with COVID-19 have been reported in the literature. The exact mechanism behind COVID-19 neurologic pathophysiology remains poorly understood due to difficulty quantifying clinical neurologic symptoms in humans and correlating them to findings in human post-mortem samples and animal models. Thus, robust preclinical experimental models for COVID-19 neurologic manifestations are urgently needed. Here, we review recent advances in *in vitro*, *in vivo*, and other models and technologies for studying COVID-19 including primary cell cultures, pluripotent stem cell-derived neurons and organoids, rodents, nonhuman primates, 3D bioprinting, artificial intelligence, and multiomics. We specifically focus our discussion on the contribution, recent advancements, and limitations these preclinical models have on furthering our understanding of COVID-19's neuropathic physiology. We also discuss these models' roles in the screening and development of therapeutics, vaccines, antiviral drugs, and herbal medicine, and on future opportunities for COVID-19 neurologic research and clinical management.



KEYWORDS: *Animal model, Cell model, Stem cell, SARS-CoV-2, COVID-19, Neurologic disorders*

COVID-19 is a viral respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first reported in Wuhan, China, in December 2019 and has since become a global pandemic.[1] There have been 768,983,095 confirmed cases of COVID-19 and 6,953,743 deaths worldwide as of August 2023 according to the World Health Organization (WHO).[2] The virus belongs to the family of coronaviruses and shares 79% genome sequence identity with severe acute respiratory syndrome coronavirus (SARS-CoV) and 50% with Middle East respiratory syndrome virus (MERS-CoV), which are two other viruses in the same virus family.[3,4] Similar to SARS-CoV and MERS-CoV, although SARS-CoV-2 is a respiratory virus, a growing number of evidence suggests that COVID-19 can cause long-term neurologic complications, even in patients with mild symptoms.[5,6] The most common COVID-19 neurologic symptoms include fatigue, myalgia, taste impairment, smell impairment, and headache.[5,7] More severe neurologic symptoms include encephalitis and stroke.[8−10] Some patients even experience "long-COVID" neurologic symptoms, having persisting symptoms 4 weeks to three months after confirmation of COVID-19 diagnosis.[11,12] The virus's neural pathophysiology has not been well understood. Given the wide range of neurologic symptoms and complications associated with COVID-19, healthcare providers need to be vigilant in monitoring patients for these clinical presentations. Further research is needed to elucidate the mechanisms by which the virus affects the nervous system and to develop effective treatments for COVID-19 neurologic conditions. Robust preclinical experimental models are urgently needed to strengthen the applicability of COVID-19 neurologic studies.

There have been recent papers published reviewing experimental models for SARS-CoV-2.[13−15] For example, Shou et al. provided a succinct overview of using animal models for SARS-CoV-2 studies, specifically studying the pulmonary and systemic pathophysiology.[14] Another paper by Bakhtazad et al. reviewed COVID-19 animal models with a more focused discussion on SARS-CoV-2 neuroinvasion mechanisms and how the pathophysiology compares to other viruses in the Coronaviruses family such as SARS-CoV, MERS-

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CoV, and murine-CoV.[15] Our paper also provides a basic overview of available COVID-19 preclinical experimental models; however, the novelty of our review is that we provide a more in-depth discussion comparing the models' applications specifically for preclinical COVID-19 neurologic studies. In addition, we discuss the significance and challenges of previous studies using these preclinical models, especially in terms of correlation with clinical symptomatology in human patients and implications for therapeutic and vaccine development. Finally, we deliberate on the newest technologies like artificial intelligence, 3D printing, and multiomics and how they can help expand the current landscape of COVID-19 neurologic studies.

1. MECHANISM OF SARS-COV-2 INFECTION

1.1. Viral Infection. SARS-CoV-2 is commonly transmitted through respiratory droplets.[16] The virus contains a single-stranded positive-sense RNA, and its envelope contains spike proteins that help the virus bind to angiotensin-converting enzyme 2 (ACE2) receptor.[3,4,17,18] ACE2 receptors are expressed in many different cell types, including epithelial cells of the oral and nasal mucosa, pneumocytes of the lung, endothelial cells of arteries and veins, intestinal mucosa cells, kidney cells, immune cells, glial cells, and neurons.[19−21] After binding with the receptor, cell proteases, mainly transmembrane protease serine 2 (*TMPRSS2*), cleave the spike protein and activate the viral membrane.[22] The viral membrane then fuses with the host cell, and the virus releases its RNA content, which is then transcribed to produce new virions.[19] The new virions then get released and infect other cells.

1.2. Neurologic Disorders of COVID-19 Infection. More than one-third of patients with COVID-19 experience neurologic symptoms during the course of their disease, and patients with more severe clinical manifestations of COVID-19 are more likely to develop neurologic symptoms and conditions.[23,24] Symptoms and conditions include dizziness, headache, acute cerebrovascular disease, epilepsy, ataxia, acute disseminated encephalomyelitis (ADEM), viral encephalitis, anosmia (loss of smell), ageusia (loss of taste), myalgia, and fatigue.[25] The mechanisms by which these neurologic symptoms and conditions develop are not well understood, and SARS-CoV-2 viral titers are only positive in a minority of patients presenting with neurologic symptoms.[26] There have been arguments for direct neurotropism of SARS-CoV-2, and there are other arguments for systemic inflammation, autoimmunity, and metabolic changes as causal factors for neurologic symptoms[27] (Figure 1).

1.2.1. Central Nervous System. Headache and dizziness are two of the most common central nervous system (CNS) symptoms and can present as isolated symptoms after COVID-19 infection.[25,28,29] Cerebrovascular accidents (CVA) like stroke are also common, especially in individuals with risk factors.[8] The mechanism behind CVA is likely the systemic inflammatory response to SARS-CoV-2 infection, such as toll-like receptor activation, endothelial dysfunction, tissue-factor pathway activation, and von Willebrand factor elevation.[30,31] Infected patients commonly develop thrombocytopenia and elevated D-dimer, a reflection of a hypercoagulable state.[32]

More severe neurologic symptoms from COVID-19 infection may manifest as encephalopathy, with clinical features such as delirium, agitation, or decreased level of consciousness.[33] These symptoms tend to be present more in hospitalized patients; for example, impaired consciousness is present in 7.5% of hospitalized patients.[34] There can be multiple underlying reasons for a patient with COVID-19 to present with altered sensorium, including viral encephalitis, metabolic perturbation, infectious toxic encephalopathy, seizures with postictal confusion, and stroke. Seizures may follow encephalopathy or may be caused by COVID-19's systemic effects.[35] The systemic release of inflammatory cytokines and colony-stimulating factors, which can trigger neuronal hyperexcitability through glutamate receptor activation, ultimately leads to seizure episodes.[36,37] In reported cases of viral meningoencephalitis, cerebral spinal fluid (CSF) analysis showed positive SARS-CoV-2 titers in some but not all patients.[9,10]

A rare form of encephalopathy, acute necrotizing encephalopathy (ANE), also has been described in the literature and is likely due to the uncontrolled release of cytokines during infection.[38] ANE leads to disruption of blood brain barrier (BBB) without direct viral invasion. Finally, there have been limited cases of ataxia and acute disseminated encephalomyelitis (ADEM) reported in COVID-19 patients.[39,40]

1.2.2. Peripheral Nervous System. Peripheral nervous system (PNS) manifestations after COVID-19 infection include anosmia, ageusia, myositis, and neuropathy. Anosmia

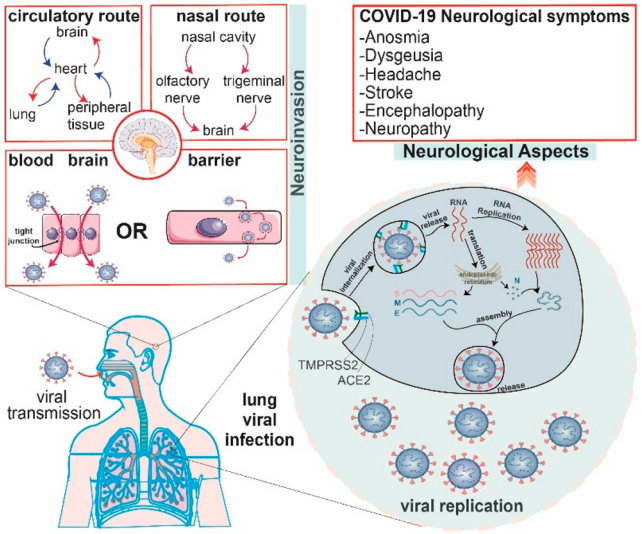

Figure 1. Proposed main mechanisms of SARS-CoV-2 virus cell entry and replication, transmission into the nervous system, and neurologic symptomatology. SARS-CoV-2 is first transmitted nasally, intratracheally, and intraocularly. In the lungs, bronchial epithelial cells have high levels of ACE2 receptors, which the virus uses to enter cells and replicate intracellularly. High levels of respiratory infection can then lead to cytokine storms and other widespread inflammation responses. This response may damage an intact blood−brain barrier, which can help viral entry into the nervous system, and the inflammatory response is associated with pro-thrombotic states, which may lead to occlusion of cerebral vessels and lead to brain injury. In addition, SARS-CoV-2 may also have direct neuroinvasive potential. SARS-CoV-2 viruses may enter nasal epithelium and neuronal cells, such as the olfactory nerve, through ACE2 receptors and then become transmitted into the brain through a neuronal retrograde fashion. SARS-CoV-2 viruses may also be able to enter the nervous system directly through other means, such as direct infection of choroid plexus cells and then dissemination through cerebrospinal fluid. As a result of the systemic inflammatory effects or direct neuroinvasion, patients develop neurologic symptoms such as anosmia and stroke. Some elements were created by Servier Medical Art.

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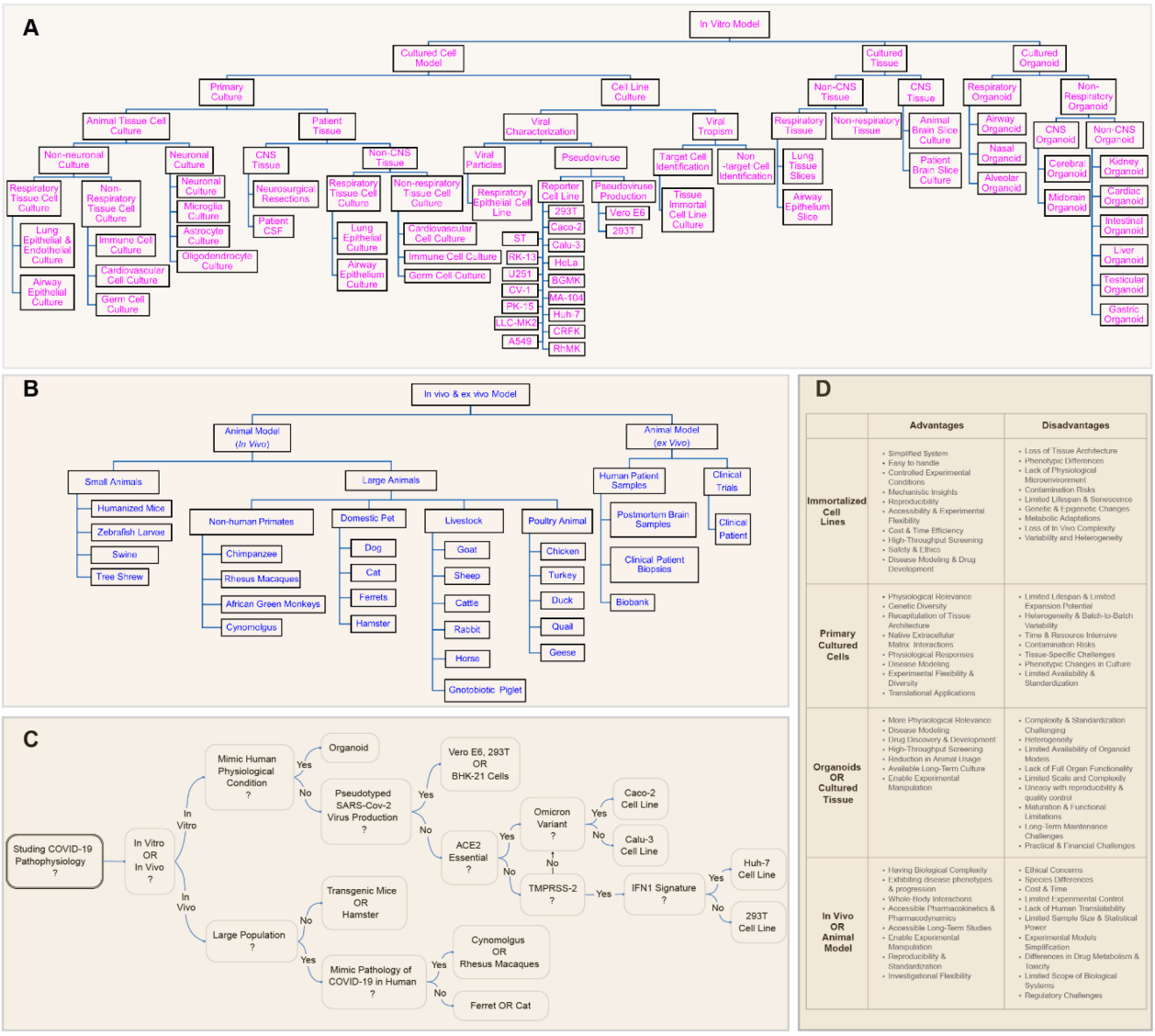

Figure 2. Common *in vitro* and *in vivo* models for studying SARS-CoV-2 viral infection and therapeutics development. (A) *In vitro* models can be constructed and studied as cells, tissues, or organoids. Common *in vitro* models are listed. (B) Common *in vivo* and *ex-vivo* models include small and large animal models and samples from patients and clinical trials. (C) Flowchart of suggested model based on the purpose and design of SARS-CoV-2 study. (D) Comparison of advantages and disadvantages of *in vitro* and *in vivo* models from a cell, tissue, or whole organism level.

and ageusia are the most common presentations, even in patients with mild COVID-19 symptoms. One study showed olfactory and gustatory dysfunctions being present in 85.6% and 88.0% of 417 patients, respectively.[41,42] Most patients slowly regain their sense of taste and smell as they recover from SARS-CoV-2 infections.[43]

Neuropathy and myositis are also complications from COVID-19 infections and can present as cranial nerve deficits, peripheral neuropathy, myalgia, and, in rare cases, Guillain-Barré syndrome.[44,45] Myalgia can present in up to one-fourth of patients after COVID-19 infection.[45] Neuropathy is a rarer presentation than myalgia, and Guillain-Barré syndrome is a severe form of neuropathy that has been reported in COVID-19 patients.[46] CSF analysis is mostly negative for SARS-CoV-2 viruses in COVID-19 patients with Guillain-Barré, which could suggest a more autoimmune pathology rather than a neurotrophic etiology.[46]

2. *IN VITRO* AND *IN VIVO* MODELS FOR INVESTIGATION OF COVID-19 NEUROLOGIC PATHOPHYSIOLOGY

2.1. Non-neuronal Cell Model. Common animal cell lines used in SARS-CoV-2 studies include Vero E6 (African green monkey kidney epithelial cells, expressing ACE2 receptor), BHK-21 (Syrian hamster kidney cells), and LLC-MK2 (rhesus macaque kidney epithelial cells). Common human cell lines include HEK 293T (human embryo kidney epithelial cells), Huh7 (human hepatocellular carcinoma), Caco2 (human epithelial colorectal adenocarcinoma cells), Calu3 (human lung adenocarcinoma), and HeLa (human cervical cell line) (Figure 2). These non-neuronal *in vitro* cell lines are used because they are generally easy to grow and become infected with SARS-CoV-2.[47−51] Notably, the HEK 293T cells are a variant of the human embryo kidney epithelial cells.[52] They express T SV-40 antigens, which enable the amplification of transfected plasmids containing the SV40 origin of replication and thus considerably increase the

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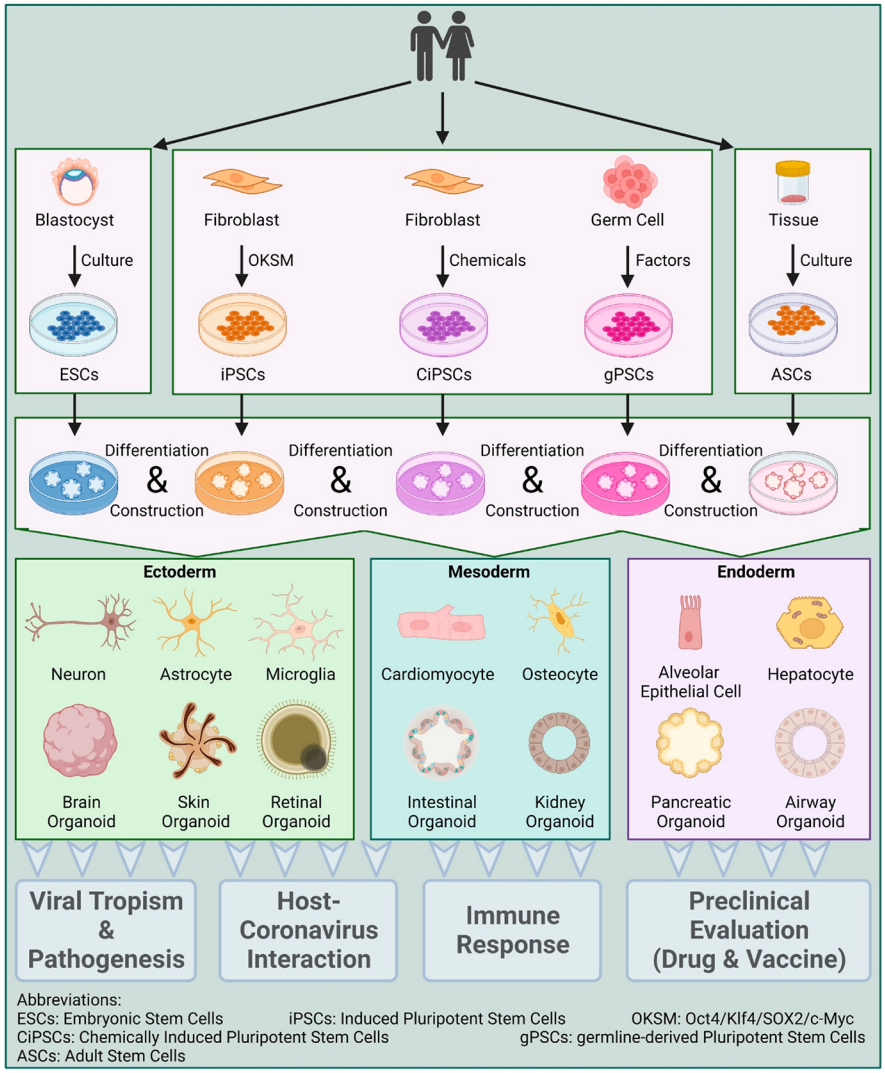

Figure 3. Organoid generation for viral pathogenesis investigation. Organoid generation can be derived from human or animal tissues or induced pluripotent stem cells (iPSCs). For tissue-derived organoids, tissue samples are first obtained from humans or animals, purified and processed, and then seeded for harvesting. For iPSC-derived organoids, iPSCs are maintained as undifferentiated populations under defined conditions and then induced to form different tissue types (ectoderm, mesoderm, or endoderm). The organoids can then be used for diverse purposes in studies, as they recapitulate *in vivo* tissue-like structures and functions *in vitro*. This figure was created with BioRender.

expression levels of desired gene products like ACE2 receptors.[52] Cell models are useful for studying viral infection and propagation mechanisms and for conducting pharmacological research.[47−51] However, most of these cell lines are behaviorally different from neural cells and often have tumor-inducing mutations, such as TP53 mutation, that may affect *in vitro* infection mechanisms and responses.[53]

2.2. PSC-Derived Neuron and Neuronal Organoid. Pluripotent stem cells are cells that can differentiate into all the derivatives of the three germ cell layers, and they are naturally present in the form of embryonic stem cells in preimplantation embryos.[54,55] Through *in vitro* technology, induced pluripotent stem cells (iPSC) are used to generate various types of neurons and neural supporting cells in a laboratory setting.[56−60] Human iPSCs-derived CNS cells have been used to study SARS-CoV-2 replicability in monolayer neuron cultures (Figure 3 and Table 1). The transcriptome of iPSC-derived cells is similar to that of their respective primary counterparts, allowing the cells to respond to immune stimuli.[61] One study observed sparse infection through immunostaining in primary astrocyte culture and hiPSC-derived cortical neurons and astrocytes but not in hiPSC-derived microglia.[62] The study revealed the ability of SARS-CoV-2 to infect monolayer human cortical neurons and astrocytes, but not microglia, although infections of neurons and astrocytes were sparse.[62] Furthermore, the study demonstrated significant productive SARS-CoV-2 viral infection, cell-to-cell viral spread, and apoptosis of primary human choroid plexus epithelial cells.[62] Another study confirmed sparse infections in hiPSC cortical neurons and strong infectability in dopaminergic neurons but found sparse infections in microglia.[63] It is unclear why the two studies showed different infectability in microglia, but different methodologies for cell preparation and inoculation may have resulted in the slightly elevated infection levels in the second study.[63] More studies would be needed to confirm the infectability of microglia cells. Other studies have similarly shown viral replicability in hiPSC neural progenitor cells, cortical neurons, and astrocytes.[64,65]

Monolayer primary and hiPSC-derived neural cells share some similar advantages with non-neuronal cell lines, including

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Table 1. SARS-CoV-2 Virus Replicability in Animal and Human Cell Lines

animal cell lines	study 1[167]	study 2[168]	study 3[169]	study 4[170]
Vero E6 (African green monkey kidney)	yes	yes, MOI of 0.001 for 1 h	yes, MOI of 0.1 for 2 h	yes
Vero81 (African green monkey kidney)	yes	yes	not tested	yes
Vero/Slam (African green monkey kidney)	yes	yes	not tested	not tested
MA104 (African green monkey kidney)	yes	not tested	not tested	not tested
BGM (African green monkey kidney)	yes	not tested	not tested	not tested
MDCK II (dog kidney)	no	yes	no	not tested
FRhK4	not tested	not tested	yes	not tested
LLC-MK2 (rhesus macaque kidney epithelium)	no	yes	yes	yes
CRFK (cat feline kidney)	not tested	not tested	yes	not tested
RK-13 (rabbit kidney)	not tested	not tested	yes	not tested
PK-15 (pig porcine kidney)	not tested	not tested	yes	not tested
human cell lines	**study 1[167]**	**study 2[168]**	**study 3[169]**	**study 4[170]**
Caco-2 (human colorectal adenocarcinoma)	significant level of virus multiplication but no cytopathic effects	yes	yes	not tested
Calu3 (human lung adenocarcinoma)	not tested	yes	yes	luciferase activity significant
293T (human embryonic kidney)	no	yes	yes	yes
Huh-7 (human hepatocellular carcinoma)	not tested	yes	yes	luciferase activity significant
A549 (human lung adenocarcinoma)	not tested	yes	no	luciferase activity significant
BEAS-2B (human bronchus normal epithelium)	not tested	yes	not tested	not tested
H1299 (human nonsmall cell lung carcinoma)	not tested	yes	not tested	not tested
U251 (human neuronal glioblastoma)	not tested	not tested	yes	not tested
HeLa (human cervical cancer)	no	not tested	no	yes

the relative ease of *in vitro* growth, infection, and therapeutic testing. There are also opportunities to generate COVID-19 patient-specific iPSCs[66] that would closely mimic individuals' neural conditions and enable personalized therapeutic approaches.[67] One limitation of *in vitro* neural cells, however, is that they do not recapitulate cellular behaviors in a 3D environment. To overcome this disadvantage, neuronal organoids can be used.

Neuronal organoids have been historically used for other viral infections that have neuro-invasive potentials, such as CMV and Zika. For example, iPSC-derived brain organoids were used to model CMV pathology similar to what was found in clinical specimens, such as an impaired generation of cortical layers.[68,69] iPSC-derived brain organoids were also used to mimic the abnormal neurogenesis process in Zika-virus infections.[70,71] Compared to viruses with well-documented neurotropism and impact on neurogenesis, the pathology behind the neurologic manifestations of SARS-CoV-2 is more complex and controversial. hiPSC-derived organoids have been used to model SARS-CoV-2 infection in many organs, including the lung, intestine, kidney, pancreas, liver, vasculature, and brain.[63,72−75] Specifically for the brain (Table 2), one study by Jacob et al. examined SARS-CoV-2 infection on hiPSC-derived cortical, hypothalamic, hippocampal, and midbrain organoids, and only sparse SARS-CoV-2 nucleoproteins were observed in these organoids (around 1%).[62] Notably, some of the hippocampal organoids contained regions with choroid plexus epithelial cells, and a greater density of infected cells was observed in those choroid plexus regions.[62] To further explore this finding in choroid plexus cells, choroid plexus organoids (CPO) expressing ACE2 and TMPRSS2 were generated.[62] After viral inoculation, CPO showed significant SARS-CoV-2 infections and replications (10−20% of the cells), inflammatory cellular responses, and cell deaths.[62] Another study used hiPSC-derived cortical organoids and choroid plexus organoids. Similarly, there were productive SARS-CoV-2 infections in choroid plexus organoids and only sparse infections were found in cortical neurons.[76] The findings in organoids are consistent with the demonstrated high infectability of monolayer culture of human choroid plexus.[62] These findings may suggest that the choroid plexus could serve as a productive infection site and act as a CNS entry site for SARS-CoV-2, leading to viral entry into the CSF. Another finding to support this theory is the high-level expression of SARS-CoV-2 entry factors, such as ACE2 and TMPRSS2, in choroid plexus compared to other brain regions.[77] Meanwhile, compared to the two aforementioned organoid studies,[62,76] a few other organoid studies[64,65,74,78] have shown higher viral proliferation in cortical neurons. More studies would be needed to confirm the infectability of cortical neurons, which would be helpful in better understanding the neurotropism of SARS-CoV-2 and thus viral entry mechanisms in the nervous system.

In addition to modeling in a 3D environment, brain organoids have the advantage of allowing analyses of SARS-CoV-2 infection in real-time, with the ability to introduce therapeutic agents.[62] A disadvantage of brain organoids is the lack of interactions with other tissue types and organs *in vitro*, such as BBB, and this is a drawback monolayer neuronal cell cultures share as well. This disadvantage results in challenges in studying the systemic mechanisms of SARS-CoV-2 infection, such as the effect of hypoxia and systemic inflammation on the CNS. One potential way to remedy this drawback is to incorporate 3D bioprinting of other cell types, such as blood vessels, into the brain organoids, which will be discussed further in a later section.

2.3. Animal Model. *2.3.1. Nonprimate Animal Model.* There is an urgent need to develop animal models that are useful for studying not only the effects of SARS-CoV-2 on the nervous system but also the systemic and long-term neurologic effects. It is important to note that no single animal model is a perfect representation of human disease, and researchers must carefully consider the strengths and limitations of each model when designing studies (Table 3). Additionally, studies in animal models must be validated by studies in humans to ensure their relevance to human disease (Figure 2).

2.3.1.1. Mouse. The mouse (*Mus musculus*) has several advantages as a virological model: low cost, ease of handling, and the possibility of large-scale studies.[79] However, wild-type and inbred strains only express the murine isoform of the

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Table 2. SARS-CoV-2 in Neural Stem Cells and Organoids in Previous Studies

ref	model	more details about the model	virus used and dose	virus expression
64	human iPSC neural progenitor cells (NPC)	iPSCs were differentiated into human neural progenitor cells for a total of 9 days	SARS-CoV-2 HKU-001a isolate from a COVID-19 patient nasopharyngeal specimen, 10 multiplicities of infection (MOI)	SARS-CoV-2 RNA gene number increased significantly at 24- and 48-h post infection (hpi); cell viability significantly decreased at 72 and 120 hpi
62	hiPSC-derived cortical neurons, astrocytes, and microglia	cortical neurons with MAP2 marker, astrocytes with GFAP, and microglia with PU1	SARS-CoV-2 USA-WA1/2020 viral isolate	120 hpi showed sparse infection of cortical neurons and astrocytes; hiPSC-derived microglia showed no infection at 48 and 120 hpi
63	hiPSC cortical neurons, microglia, and dopaminergic neurons	day 45 cortical neurons with beta III-tubulin, dopaminergic neurons with FOXA2 and MAP2, microglial cells with PU.1 and IBA1	vesicular stomatitis virus (VSV)-based SARS-CoV-2 pseudoentry virus, inoculation for 24 h	dopaminergic neurons but not microglia or cortical neurons were infected (measured by pseudovirus reporter luciferase activity)
65	iPSC culture NPCs, neurons, and astrocytes	NPCs with SOX-2, cortical neurons with MAP-2, and astrocytes with GFAP	SARS-CoV-2 USA-WA1/2020 isolate, 2 MOI	at 2 and 48 hpi, all three cell types supported viral replication over time, with the amount of viral RNA being highest in neurons when compared with NPCs and astrocytes
62	hiPSC-derived organoids	cortical, hippocampal, hypothalamic, and midbrain (CTIP2, PROX1, OTP, and TH as markers, respectively); mixed cell types (e.g., neurons and astrocytes)	SARS-CoV-2 USA-WA1/2020 viral isolate	viral nucleoprotein was detected sparsely in the organoids in a range that averaged between 0.6% and 1.2% of all cells at 24 and 72 hpi; most of the infected cells are neurons
64	brain organoids	35-day-old brain organoid, mixed cell types resembling developing cerebral cortex (e.g., neural progenitor cells)	SARS-CoV-2 HKU-001a from a COVID-19 patient nasopharyngeal specimen	extensive SARS-CoV-2 antigen was detected at 72 hpi; infection colocalized with neuronal marker
76	brain organoids	organoids aged more than 55 days; mixed cell types, (e.g., choroid plexus and cortical tissue)	SARS-CoV-2 spike pseudo virions carrying a 19-amino-acid deletion (c19) from the C terminus	significant increase in viral genome copies in organoid supernatant between 24 and 48 hpi; around 13% of cells in the choroid plexus were infected, and neuronal regions mostly did not get infected
65	brain organoids	mixed cell types, such as NPCs, neurons, and glial cells characterized by their respective markers SOX-2, MAP-2, and GFAP	SARS-CoV-2 pseudo virus, 2 MOI	infection was more efficient in neurons than in NPCs at 24 hpi
74	brain organoids	two different age groups of organoids (day 15 and day 60)	SARS-CoV-2 (NRW-42) isolate from a nasopharyngeal and oropharyngeal swab specimen of an infected patient, TCID50/mL of 50 or 17.5 PFU/organoid	at 48 and 96 hpi, day-60 organoid had a significantly higher number of viral-positive cells than day-15 organoid
78	brain organoids	9-week-old organoids	SARS-CoV-2 isolate USA-WA1/2020	at 24 and 96 hpi, there was a significantly increasing number of SARS-CoV-2 positive cells; many infected cells were MAP2-positive mature neurons, but sparse infections of SOX2-positive neural stem cells were also observed; at 96 hpi there was a widespread infection, mostly limited to the regions with high cortical cell density
62	choroid plexus organoid	47 and 67 days *in vitro*	SARS-CoV-2 USA-WA1/2020 viral isolate	many TTR+ choroid plexus cells showed infection

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Table 3. SARS-CoV-2 Infectivity and Pathologic Response of Different Species of Animal Models

animal model	ACE2 receptors	neurotropism	pathophysiology of SARS-CoV-2 infections	ref
wild type mice	have murine isoform of ACE2 receptor, but it has a low affinity for viral S protein on SAR-CoV-2; modified SARS-CoV-2 strains with higher affinity to murine ACE2 receptor, such as SARS-CoV-2 B.1.351 and SARS-CoV-2 MA, are needed to produce disease states in both young and old mice	not determined	modified SARS-CoV-2 strains produced lung abnormalities; SARS-CoV-2 MA caused more severe disease in older mice	80, 171−173
K18-hACE2 transgenic mice	transgenic human ACE2 receptor expressed through K18 promoter	increased viral titer after intranasal administration measured by viral nucleocapsid protein antigen	local and systemic elevation of chemokines, including the brain; elevated levels of IFN-λ in the lungs; tissue pathology including vasculitis is shown in the brain; perivascular inflammation is observed	78, 82, 84, 174−175
HFH4-hACE2 transgenic mice	transgenic human ACE2 receptor expressed through HFH4	high levels of virus and ACE2 receptors were detected in the brain	severe immune and pathological response like K18-hACE2 mice	82, 83, 172
Syrian hamster (*Mesocricetus auratus*)	Syrian hamster ACE2 receptors are homologous to humans	virus or viral RNA in the CNS and neurovascular inflammation have been detected by some studies; retrograde infection from olfactory infection may be possible	mononuclear cell infiltrates, apoptosis, and pro-inflammatory cytokines like IFN-γ are observed in the lungs and airways early after infection (2−3 days)	100, 102−104, 176, 177
ferret	ferret ACE2 receptors share some amino acid residues with humans	no, but is present in olfactory tissues, such as the nasal turbinate and sustentacular cells of the olfactory epithelium	virus multiplication and antibodies are detectable in the upper respiratory tract a few days after exposure	109, 178−180
nonhuman primates (gibbon, green monkey, macaque, orangutan, and chimpanzee)	ACE2 receptors have identical sequences in regions important for interaction with SARS-CoV-2	mixed evidence; some studies demonstrate neuronal damage due to systemic effects, such as strokes due to hypercoagulable states, but no viral level in CSF; other studies show high viral in the brain and olfactory bulb	lung infiltrates and elevated serum cytokine levels (cytokine storm); decreased hematocrit and hemoglobin; rhesus macaques have more severe disease than other macaques; aged primates have more severe disease	109−112, 116− 119, 178, 181−182

ACE2 receptor, to which the SARS-CoV-2 virus has low binding affinity.[80] To overcome this barrier, SARS-CoV-2 studies have used transgenic mice expressing human ACE2 (hACE2).[81]

K18 (epithelial cell cytokeratin-18 promoter) is an exogenous promoter that can be used to induce transgenic hACE2 expression in mice.[82,83] It was originally developed for studying SARS-CoV and subsequently evaluated for SARS-CoV-2.[84] Song et al. used K18-hACE2 transgenic mice and observed increasing viral titers in mice brains after intranasal administration of SARS-CoV-2.[78] The virus was widely present in neural cells in the forebrain but was not detected in the cerebellum, except in the pial meninges and dorsal cochlear nucleus. Other regions also contained a relatively low density of infected cells, the dentate gyrus, the globus pallidus, and cortical layer 4.[78] Notably, the virus was not detected in the vascular endothelium.[78] Compared to SARS-CoV-2 studies, SARS-CoV studies using K18 transgenic mice were more lethal and more symptomatic.[85,86]

Hepatocyte nuclear factor-3/forkhead homologue 4 (HFH-4/FOXJ) is another exogenous promoter, expressed in developing mice cells' lung motile cilia.[81] Transgenic mice expressing HFH4-hACE2 have been shown to be susceptible to SARS-CoV-2 infection.[81] HFH4-hACE2 transgenic mice demonstrated brain infection after intranasal administration of the SARS-CoV-2 virus.[87,88] Only ∼60% of mice survived after infection, and interestingly, significant brain viral levels were only detected in the deceased mice.[87,88] The specific brain regions that were infected were not discussed.[87,88]

Adenovirus and adeno-associated virus (AAV) are vector options to deliver exogenous promoters in mice. Compared with adenoviral vectors, the use of AAV tends to elicit less host immune response.[89] However, one common drawback for both the use of adenovirus and AAV is the possibility of nonuniform viral transduction and thus expression in the neural tissues. Using AAV virus, Song et al. compared respiratory versus neural routes of administration on SARS-CoV-2 neuroinvasion. Intratracheal administration of AAV virus to express hACE2 receptors followed by intranasal administration of SARS-CoV-2 (respiratory route) was compared with intracisternal AAV and intraventricular SARS-CoV-2 administration (neural route). Intranasally infected mice showed signs of lung pathology but no weight loss or death. However, intraventricularly infected mice showed weight loss and death, even at the challenge virus dose 100-fold lower than that used for intranasal infection.[78] This result highlights the potential of SARS-CoV-2 for neuroinvasion through the hACE2 receptors and causing lethality in AAV-hACE2 mice. In addition, Song et al. demonstrated that using AAV induction, hACE2 can be induced in an organ-specific manner.

Some newer transgenic hACE2 mouse models have also been generated by CRISPR-Cas9 and knock-in approaches, thus endogenously expressing human ACE2 receptors. These mouse models with endogenous hACE2 expression may be more clinically similar to humans than the mouse models expressing exogenous genetic materials.[90] Intranasal administration of SARS-CoV-2 in CRISPR-generated hACE2 mice has demonstrated significant viral load in the lung and brain.[91]

One common drawback of all the discussed transgenic mouse models is that unlike in what is naturally seen in human neural tissues, hACE2 is highly expressed in these mice's nervous system, which may misconstrue the infection

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mechanisms and presentations.[83,84,92,93] Another drawback is the length of time needed to develop these mouse strains.

In short, the need to engineer human ACE2 expression is a limitation of the mouse model because hACE2 receptors in mice may not be expressed in the same cell types and at the same levels as they are in humans. This may lead to higher levels of SARS-CoV-2 neuro-invasion in hACE2 transgenic mice than in the native human nervous system. On the other hand, the silver lining is that transgenic mouse models can show which cell types allow SARS-CoV-2 neuro-invasion when expressing hACE2 receptors, allowing researchers to test mechanisms of neuro-invasion as well as antiviral strategies.[94,95] Another advantage of using hACE2 mice is the ability to study long-COVID and its correlation with different acute-phase infection severities. By adjusting hACE2 expressions in mice, researchers can create mouse models of mild infections and see if they develop long-COVID symptoms. This may be important in studying long COVID, as young patients who have only had mild symptoms during the acute infection phase can still develop long-COVID symptoms that impact their quality of life.[96] For a more in-depth review of the implications of animal studies on studying the neural impact of long-COVID, Jansen et al. provide a thorough overview and discussion.[97]

2.3.1.2. Hamster. Golden Syrian hamsters (*Mesocricetus auratus*) have been used extensively for virological studies, such as for SARS-CoV and influenza virus.[98] In a systematic review of hamsters, seven studies investigated whether the virus was present in the brain following SARS-CoV-2 nasal inoculation.[99] Three studies found either viral RNA or virus (by plaque formation) in the olfactory bulb or brain at 1 to 14 days postinfection,[100−102] with 2 logs lower than in nasal turbinate and with similar levels in the brainstem, cerebral cortex, and cerebellum.[102] The other four studies found no evidence of brain infection with antibodies. One advantage of using hamsters for SARS-CoV-2 studies is that the hamster ACE2 gene is highly analogous to human ACE2 and that hamsters can be infected by and transmit SARS-CoV-2.[98,103,104] Hamsters exhibit viral pathogenesis and immune responses consistent with those seen in humans.[98] While hamsters are easy to handle, the availability of hamster-compatible reagents, such as reagents to examine immune responses and protein expression, is limited.[100]

2.3.1.3. Ferret. Ferrets (*Mustula putorius furo*) are another commonly used animal model because of their ability to transmit human respiratory viruses.[105,106] In addition to the presence of appropriate viral receptors, histo-anatomical features of the ferret respiratory tract are analogous to that of the human respiratory tract.[107,108] Another advantage of ferrets is their larger size than most other rodents. This allows for more volume of blood tests for immunological analyses and monitoring.

In terms of neural studies, SARS-CoV-2 viral RNA has not been found in the brain of ferrets after intranasal administration but is present in olfactory tissues, such as the nasal turbinate and sustentacular cells of the olfactory epithelium.[109] These findings are consistent with those of hamsters.[100−102]

2.3.1.4. Others. Domestic animals such as pigs, dogs, chickens, and ducks may not be good models for studying COVID-19 neurologic effects because they have low susceptibility to SARS-CoV-2 infections. Serum and organ samples of pigs, dogs, chickens, and ducks show low levels of SARS-CoV-2 replication after intranasal viral inoculation.[110]

2.3.2. Nonhuman Primate Animal Model. Nonhuman primates (NHPs) are phylogenetically close to humans, thus are generally beneficial for studying infections. NHPs including rhesus macaques (*Macaca mulatta*),[111−114] cynomolgus macaques (*Macaca fascicularis*),[113,115] African baboons (*Papio hamadryas*),[114] and African green monkeys (AGMs) (*Chlorocebus aethiops*).[116] NHPs have been demonstrated to be permissive to SARSCoV-2 infection and present symptoms similar to humans such as fever, diarrhea, and pneumonitis.[111−115]

Studies using NHPs have generated mixed results about whether there are detectable viral loads in different CNS regions. For example, one study by Munster et al. showed that in infected rhesus macaques presenting with respiratory symptoms, no significant SARS-CoV-2 viral loads were detected in frontal lobes, brainstems, or cerebellum.[117] Another study done by Rutkai et al. confirms the lack of direct cortical infection and provides an explanation for the route of neurologic manifestations in NHPs.[118] Rutkai et al. used Rhesus macaques and African green monkeys to demonstrate neuronal injury, microhemorrhage and strokes, and brain hypoxia after mucosal SARS-CoV-2 infection. Suspected endothelial cell infection was confirmed by colocalization immunofluorescence, but SARS-CoV-2 virus was not identified in the CSF, consistent with most findings among human subjects except in rare cases of encephalitis.[118] There were elevated CO_2 levels in the animals, suggesting mild hypoxemia and impaired gas exchange in the lungs.[118] On the basis of this, Rutkai et al. concluded that the underlying cause of neuronal injury was a lack of oxygen.[118] This does not appear to be a direct consequence of virus infection, as only limited virus was seen in brain vasculature and did not appear to involve parenchymal cells.[118] Instead, neuronal injury and death most likely occur because of energy failure, which is an early consequence of hypoxic-ischemic events.[118]

On the contrary, Jiao et al. found evidence of virus RNA and nuclear capsid antigen in the brain of NHPs, including the olfactory bulb, suggesting direct neuro-invasion of SARS-CoV-2.[119] However, this study applied an extremely high dose of the virus, about 20 times higher than the other monkey studies.[119] Thus, more studies and caution are needed to confirm SARS-CoV-2 neuro-infectability of NHPs.

3. VACCINE AND THERAPEUTICS DEVELOPMENT USING PRE-CLINICAL MODELS

When considering preclinical models for vaccine and therapeutic development, the advantages and disadvantages discussed in earlier sections should be taken into consideration. For example, mice can be used for rapid screening of vaccines, antivirals, and other therapeutics in a pipeline high output approach. However, mice's structural difference in their ACE2 receptors from human ACE2 receptors results in reduced susceptibility to SARS-CoV-2 infection and thus more difficulty in testing the effectiveness of vaccines and therapeutics. Using hACE2-transgenic mice can be a remedy to the shortcoming as discussed previously. The National Institutes of Health (NIH) Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) Preclinical Working Group has developed animal model summaries and descriptions for studying vaccines and therapeutics that may be helpful for reference.[120] A reoccurring theme is that SARS-CoV-2 can infect certain species like nonhuman primates, hamsters, ferrets, and hACE2 transgenic mice, but clinical

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disease and symptomatology in these models have generally been mild (Table 4). It is important to consider the difference

Table 4. SARS-CoV-2 Symptoms Comparison between Human and Animal Models

neuronal and non-neuronal symptoms	human	animals	ref
increased body temperature	yes	rhesus macaque, cynomolgus macaques, and ferret	183 and 184
chest radiograph abnormality	yes	rhesus macaque and cynomolgus macaques	184
coughing	yes	rhesus macaque	117
reduced appetite	maybe	rhesus macaque	117
weight loss	maybe	rhesus macaque, Syrian hamster	102 and 117
dysgeusia	yes	Syrian hamster	102
hyposmia/anosmia	yes	Syrian hamster, hACE2 mice	95, 102, and 185
fatigue	yes	Syrian hamster	103 and 186
other neurologic symptoms	yes	unclear	

in nature and severity of symptoms between what the animal models capture and what is present in human patients when selecting models for studies.

3.1. Vaccine. There are currently three major vaccines approved by the US Food and Drug Administration (FDA) for emergency use in the US, and each of them has gone through extensive and vigorous preclinical testing using cellular and animal models. BioNTech/Pfizer and Moderna vaccines utilize a novel mRNA (mRNA) approach, in which mRNA encoding a subunit of the SARS-CoV-2 spike protein is delivered with a lipid nanoparticle encapsulation to increase stability and uptake by antigen presenting cells.[121] The mRNA is then translated into spike protein, inducing cell and humoral immunity.[121] The Johnson & Johnson vaccine, in contrast, uses a replication-deficient adenovirus vector to deliver spike-protein DNA into intracellular space, which then is made into antigenic protein.[122]

The developers of the BioNTech/Pfizer vaccine (BNT162b2 mRNA) used cells, mice, and nonhuman primates for their preclinical data.[121] HEK293*T*/17 and Expi293F cells were used to test expression levels and structural characteristics of the SARS-CoV-2 spike protein from mRNA incubation.[121] Following BNT162b2 transfection, BALB/c mice were used to detect and characterize the immunogenicity of the vaccine. The authors found that BNT162b2 elicits strong virus-neutralizing titers and systemic T cell responses.[121] Moderna also similarly used mice, specifically BALB/cJ, C57BL/6J, and B6C3F1/J mice, and demonstrated effective immunogenicity of their mRNA-1273 vaccine.[122] Finally, BioNTech/Pfizer used 2- to 4-year-old rhesus macaques to detect antibody development and protection from SARS-CoV-2 infection challenge tests.[121] The authors found that vaccination significantly reduced virus levels found in nasal and oropharyngeal swabs and bronchoalveolar lavage.[121] Notably, the authors commented that they found no clinical signs of significant disease in virus-challenged nonhuman primates (rhesus macaques).[121] Vaccine data from nonhuman animal models must be interpreted with caution, especially from a symptomatology perspective. What is reassuring, however, is the recent data showing that mild clinical manifestations of COVID-19 in macaques, measured by CT scan and histopathological samples, are mostly similar to that seen in humans.[123]

The Johnson & Johnson Ad26 vaccine was tested on hamsters and rhesus macaques.[124] In hamsters, the authors demonstrated that a single immunization with an Ad26-vector-containing spike-protein DNA induced immunogenicity and antibody formation, which protected against severe clinical diseases after the high-dose SARS-CoV-2 challenge.[124] The authors chose hamsters as a preclinical model because hamsters could be used to generate severe pneumonia models mimicking clinically severe infections in humans.[125] The authors also studied the vaccine on rhesus macaques and showed that a single immunization of their vaccine induced a strong neutralizing antibody response and provided protection against the SARS-CoV-2 challenge, similar to the results in hamsters.[124]

Vaccines have been shown to be effective in preventing severe COVID-19 infection in human clinical data. For example, the BioNTech/Pfizer vaccine had 95% efficacy in preventing infection and symptom development.[121] Neurologic symptoms assessed included myalgia, anosmia, and ageusia.[126] Furthermore, vaccination has been shown to reduce stroke incidence after COVID-19 infection.[126] This is logical because if vaccines can prevent severe systemic inflammation, vaccines would likely have protective effects against COVID-19 neurologic symptoms. However, there have been controversies about rare cases of serious neurologic side effects from COVID-19 vaccination, possibly due to an autoimmunity etiology. For example, in Fall 2020, two patients developed transverse myelitis after receiving the Oxford/AstraZeneca vaccine.[127,128] In another study of the mRNA vaccines by BioNTech/Pfizer and Moderna, 7 cases out of 37,000 vaccine recipients developed Bell's palsy, though the FDA commented that the indicated incidence of Bell's palsy was not higher than expected in the general population.[129]

3.2. Therapeutics. Commonly used drugs to treat COVID-19 include antiviral (remdesivir, Molnupiravir), anti-inflammation (dexamethasone, Baricitinib), anticoagulability, and immunoglobulin drugs (convalescent plasma). Most of these drugs have been shown in clinical trials to reduce the severity of COVID-19 infection and lower the rate of hospitalizations.[130−132] Currently, the FDA (Food Drug Administration) has approved tocilizumab, remdesivir, baricitinib, nirmatrelvir, and ritonavir.[133]

By inference, if these drugs can reduce the severity of COVID-19 infection, they likely reduce COVID-19 neurologic symptoms, as COVID-hospitalization has been shown to be a risk factor for COVID-19 neurologic symptoms.[134] Indeed, Grundmann et al. have shown that treatment with dexamethasone and remdesivir is associated with reduced mortality and lower frequency of neurologic complications, notably stroke, seizure, and meningitis.[135] Grundmann et al. also showed that combined dexamethasone and remdesivir resulted in a further reduction of neurologic complications than single-drug therapy.[135] More studies, both clinical and preclinical, are needed to investigate the mechanisms behind neurologic COVID-19 drug complications and to discover new or off-brand use of drugs. For example, Baricitinib was originally indicated for alopecia and rheumatoid arthritis but has recently been approved for COVID-19 use.[136] Preclinical neurologic studies of COVID-19 drugs are especially needed; currently limited studies include that of Wang et al. (2021), which found that the antiviral drug remdesivir effectively inhibited SARS-

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CoV-2 infection in hiPSC-derived neurons and resulted in improvement of neurite lengths of infected neurons.[137]

Another area of opportunity for COVID-19 therapeutics is herbal medicine. For instance, prior to the COVID-19 pandemic, the traditional Chinese medicine Lianhuaqingwen was shown to be effective in treating influenza.[138,139] Then during the pandemic, there have been clinical trials and case-control studies on Lianhuaqingwen for SARS-CoV-2 infection.[140−144] A meta-analysis of these studies showed that Lianhuaqingwen can significantly improve clinical COVID-19 symptoms and slow progression to severe conditions from infection.[145] Another systematic review showed that Lianhuaqingwen combined with conventional treatment (e.g., oxygen therapy and antiviral medications) is more effective in treating mild or moderate COVID-19 clinical symptoms than conventional treatment alone.[146] However, both the meta-analysis and the systematic-review studies caution about the quality of the included studies, thus more studies are needed to confirm the efficacy of Lianhuaqingwen.[145,146] Other herbal medicines have also shown clinical potential, and Alam et al. have provided a comprehensive review and discussion on currently available herbal medicines for COVID-19 and their supporting studies.[147]

4. PERSPECTIVE

4.1. Future Directions for Model Generation and Improvement. FDA approves only around 10% of neurology drugs from phase I clinical studies.[148] A major reason hindering the success of drug development is the preclinical models' lack of recapitulation of human pathophysiology. Therefore, there is a strong need for robust modeling of human neurologic diseases.

One direction for future COVID-19 model generation is developing more sophisticated brain organoid models (Figure 3). For example, Gleeson et al. integrated perivascular cells into cortical organoids and found that these organoids had more extensive SARS-CoV-2 infection than the traditional organoids.[149] More studies using such "assembloid" models with crosstalk between different tissue types are underway. Future studies can expand the single-organoid model to a multiorgan system by connecting the brain organoid to other organs such as lung organoid[63] and cardiac organoid[150] through blood circulation. Doing so would allow better modeling of systemic responses to COVID-19 infection, such as inflammation and hypoxia, at the whole organism level and the impact on the nervous system. Another avenue of opportunities is using organoids derived from cells of patients at different stages of COVID-19 infection to draw correlations between pathophysiology and clinical findings.

To facilitate the development of more sophisticated organoids, 3D bioprinting is a technology with great potential. 3D bioprinting offers a platform to address the challenges of integrating multiple cell types in brain organoids. 3D bioprinting allows precise placement of cells and growth factors through a bioprinter on a building block like an organoid.[151] A recent study by Ahn et al. developed a microengineered physiological system to model the BBB.[152] Their platform contained two compartmental microfluidic channel layers.[152] The upper layer is a porous membrane with monolayer endothelial cells above and pericytes below the membrane.[152] The lower layer contained astrocytes in a 3D Matrigel matrix.[152] The authors reported features of this model resembling native BBB, including low permeability and similar architectural features.[152]

Another challenge is mimicking the human systemic immune response to COVID-19, such as cytokine storms. One solution is to design immune-competent humanized models. For instance, humanized mice have been made by implanting xenografts of human lung tissue into immunodeficient mice, producing bone marrow/liver/thymus-lung (BLT-L) mice.[153] These mice then demonstrated infectability with RSV virus, Zika virus, MERS-CoV, and cytomegalovirus and produced an immune response mimicking humans.[153] Challenges of making humanized models include the technical expertise required, long preparation time, and immunocompromised status of the animal models.

Finally, a possibility for the future is xenotransplantation of human donor organs into pigs. Pigs have many similarities with humans in organ systems and size.[154] Though there have not been actual cases of transplantation into pigs, the idea comes from recent clinical cases of pig heart and kidney transplantation into humans.[155,156] If pig organs can be transplanted into humans, doing so vice versa may also be a possibility. These pig models with humanized organs would more accurately capture neurologic COVID-19 pathophysiology and symptomatology. These models can offer new perspectives that include demonstrating the relationship between infection severity and symptoms and comorbidities such as type 1 diabetes, hypertension, and obesity. Nonetheless, caution certainly must be taken with pursuing this idea, as there would be ethical concerns and public health concerns about zoonotic diseases.

4.2. Artificial Intelligence-Based Drug Discovery. New CNS drugs on average take 15−19 years to advance from discovery to regulatory approval.[157] This would be too slow for a new emerging disease causing a global health crisis like COVID-19. Therefore, models and technologies to facilitate a swift generation of effective therapeutic targets and agents would be essential. With the tremendous recent advancements in machine learning (ML), artificial intelligence (AI) shows great potential for facilitating drug discovery and application (Figure 4). Furthermore, taking advantage of AI techniques for modeling COVID-19 pathology can replace, reduce, and refine the use of animal models in studies,[158] achieving more humane approaches while ensuring high-quality outcomes.

In terms of drug discovery, AI/ML has been used at three different early stages of the drug discovery process−target identification, lead generation and optimization, and preclinical development.[159,160] In target identification, AI-based approaches have been used to help elucidate molecular mechanisms and pathways underlying diseases and drug activities.[159] For lead generation and optimization, algorithms improve the functions and quantitative structure−activity relationship (QSAR) models in screening pipelines and help automate and optimize drug design *de novo*.[159] In preclinical development, AI/ML approaches are utilized to predict physical-chemical properties by processing large amounts of data and to optimize pharmacokinetics and pharmacodynamics.[159]

An example of using AI to discover off-brand uses of approved medicine is halicin in early 2020.[161] Researchers at MIT trained a deep graph-convoluted network (GCN) model based on drug candidates' molecular features from the Drug-Repurposing Hub, an open-access repository of more than 6,000 compounds many of which the FDA has approved.[161]

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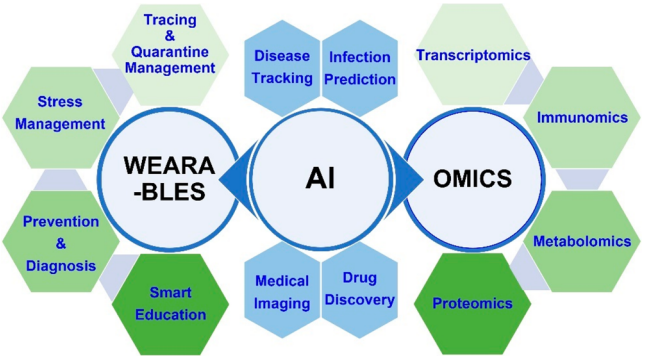

Figure 4. Artificial intelligence application in the COVID-19 pandemic. AI provides a unique opportunity to simultaneously advance infection prevention and management strategies for individual patients and link them to understanding the virus from a population level. For example, studies on "omics" like proteomics and metabolomics can help identify abnormal biomarker levels in infected individual patients and help guide management strategies. Wearable technologies on individuals can also help identify signs (such as elevated body temperature) of infection and offer biophysical and psychological feedback on coping with infections or concerns about infections. Compiling and analyzing these data from individual patients (with consent from users) can then help research agencies identify patterns on prevention/quarantine/virus spread, drug development, and management strategies on a population level using AI algorithms.

Using the GCN, the team identified halicin, originally labeled as a diabetes drug, as a new potential antibiotic.[161] Then, halicin showed a broad-spectrum activity against drug-resistant strains in mice.[161] This was the first time an AI/ML-assisted tool was used to identify a new type of antibiotic from scratch. This goes to show that the AI/ML technology holds immense potential for new emerging diseases like COVID-19 and could reduce the burden of animal model usage. Furthermore, with enough data volume, such as a large registry of neurologic symptoms and treatment outcomes from hospitals and clinics, robust AI/ML algorithms can be developed and used to generate management strategies based on each patient's individualized clinical picture.

4.3. Multiomics in Unraveling the Landscape of SARS-CoV-2 Infection. Given recent advancements in sequencing technologies, access to large-scale omics data sets (e.g., genomics, transcriptomics, proteomics, metabolomics, metagenomics, phenomics) could help revolutionize our understanding of SARS-CoV-2 and its effects on the nervous system.[162] With the help of machine learning, multiomics studies can be done to identify neurologic biomarkers that can be used to diagnose and monitor disease progression. For example, one study collected patient blood serum and used proteomics and metabolomics to identify molecular differences from the non-COVID-19 population—dysregulation of macrophage, platelet degranulation, complement system pathways, and metabolic suppression.[163] Omics can also help classify the morphological observations underlying COVID-19 neurologic pathology. For example, one study used proteomics to characterize over 10,000 proteins in organ autopsy samples and identified multiple dysregulated proteins involved in fibrosis, coagulation, hypoxia, angiogenesis, and glucose and fatty acid metabolism.[164] As more studies in proteomics and metabolomics are done, there may be recurring findings of certain protein and metabolite dysregulation involved in the pathology of patients with neurologic symptoms. Using AI and sampling a large population of patients across different spectra of symptoms and severity, the morphological and metabolic targets can be used to develop and monitor COVID-19 neurologic disease prognostics, progression, therapeutics, and prevention strategies[165,166] (Figure 4).

5. CONCLUSIONS

Neurologic complications of SARS-CoV-2 infection are common, and a better understanding of neurologic pathophysiology is urgently needed for more effective management and treatment strategies. In this paper, we discussed available preclinical models and their pros and cons for research on disease etiology, vaccines, and therapeutics. We also presented new models and opportunities in the field of COVID-19 neurologic research. This paper has focused on addressing the acute phase of neurologic COVID-19; an area of future discussion is "long-COVID" after the acute phase of infection, which is also highly prevalent.

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Notes

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