Drug discovery strategies for SARS-CoV-2

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List of abbreviations:

ACE
Angiotensin converting enzyme

ADE
Antibody-dependent enhancement

ARDS
Acute respiratory distress syndrome

BSL
Biosafety level

3CLpro
3C-like serine protease

COVID-19
Coronavirus disease 2019

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EBOV
Ebola virus

EndoU
Uridine-specific endoribonuclease

FDA
Food and Drug Administration

HIV
Human immunodeficient virus

MERS-CoV
Middle east respiratory syndrome coronavirus

NSP
Non-structural protein

RBD
Receptor binding domain

RdRp
RNA dependent RNA polymerase

SARS-CoV
Sudden acute respiratory syndrome coronavirus

S protein
Spike protein

TMPRSS2
Transmembrane protease serine type 2

Abstract

COVID-19 is a novel disease caused by the SARS-CoV-2 virus that was first detected in December of 2019 in Wuhan, China and has rapidly spread worldwide. The search for a suitable vaccine as well as effective therapeutics for the treatment of COVID-19 is underway. Drug repurposing screens provide a useful and effective solution for identifying potential therapeutics against SARS-CoV-2. For example, the experimental drug remdesivir, originally developed for Ebola virus infections, has been approved by the FDA as an emergency use treatment for COVID-19. However, the efficacy and toxicity of this drug needs further improvements. In this review, we discuss recent findings on the pathology of coronaviruses and the drug targets for the treatment of COVID-19. Both SARS-CoV-2 specific inhibitors and broad-spectrum anti-coronavirus drugs against SARS-CoV, MERS-CoV, and SARS-CoV-2 will be valuable additions to the anti-SARS-CoV-2 armament. A multi-target treatment approach with synergistic drug combinations containing different mechanisms of action may be a practical therapeutic strategy for the treatment of severe COVID-19.

Significance Statement

Understanding the biology and pathology of RNA viruses is critical to accomplish the challenging task of developing vaccines and therapeutics against SARS-CoV-2. This review highlights the anti-SARS-CoV-2 drug targets and therapeutic development strategies for COVID-19 treatment.

Introduction

Coronaviruses are enveloped, single-stranded, positive-sensed RNA viruses belonging to the family *Coronaviridae* with genomes ranging from 26 to 32 kilobases in length. Several known strains of coronaviruses such as OC43, HKU, 229E5, and NL63 are pathogenic to humans and associated with mild common cold symptoms (Gordon et al., 2020). However, in the past two decades, three notable coronaviruses of the pandemic scale have emerged and produced severe clinical symptoms including acute respiratory distress syndrome (ARDS). In 2002, the coronavirus strain SARS-CoV, named for causing severe acute respiratory syndrome (SARS), originated in the Guangdong province of China (Drosten et al., 2003). In 2012, another coronavirus with reported clinical similarity to SARS-CoV was first detected in Saudi Arabia, and later identified as Middle East respiratory syndrome coronavirus (MERS-CoV) (Zaki et al., 2012). SARS-CoV resulted in more than 8000 human infections and 774 deaths in 37 countries between 2002-2003 (Lu et al., 2020) before disappearing from the population due to stringent quarantine precautions. MERS-CoV infections, however, are a continued threat to global health. Since September 2012, there have been 2494 laboratory-confirmed cases and 858 fatalities, including 38 deaths following a single introduction into South Korea (Lu et al., 2020). Despite significant efforts, vaccines or effective drugs for the prevention or treatment of either SARS-CoV or MERS-CoV are still not available.

In December 2019, a new virus initially called the 2019 novel coronavirus (2019-nCoV) emerged in the city of Wuhan, China. It produced clinical symptoms that included fever, dry cough, dyspnea, headache, pneumonia with potentially progressive respiratory failure owing to alveolar damage, and even death (Zhou et al., 2020). Because sequence analysis of this novel coronavirus identified it as closely related to the SARS-CoV strain from 2002-2003, the World Health Organization (WHO) renamed the new virus as SARS-CoV-2 in February 2020. The disease caused by SARS-CoV-2 has been named coronavirus disease 2019 (COVID-19). Like

SARS-CoV and MERS-CoV infections, ARDS can be induced in severe cases of COVID-19. ARDS is largely mediated through the significant release of pro-inflammatory cytokines that results in a cytokine storm, which likely triggers multi-organ failure and contributes to increased death rates (Li et al., 2020). Dependent on several factors such as pre-existing conditions and the immune response, severe disease can precipitate pathophysiological effects on the heart, kidney, liver and the central nervous system. Examples include myocardial injury, arrhythmias, increased risk of myocardial infarction, liver dysfunction, kidney failure, neurological complications such as ataxia, seizures, neuralgia, acute cerebrovascular disease and encephalopathy (See (Zaim et al., 2020) for an in depth review). In addition, SARS-CoV-2 may have tropism towards tissues other than the lungs, which could contribute to disease exacerbation (Puelles et al., 2020).

Genome sequencing and phylogenetic analyses have confirmed that SARS-CoV, MERS-CoV and SARS-CoV-2 are all zoonotic diseases that originated from bat coronaviruses leading to infections in humans either directly or indirectly through an intermediate host (Lu et al., 2020). Unfortunately, predicting the zoonotic potential of newly detected viruses has been severely hindered by a lack of functional data for viral sequences in these animals (Letko et al., 2020). Unlike SARS-CoV or MERS-CoV, where transmissions mainly occur in a nosocomial manner, SARS-CoV-2 appears to spread more efficiently as viral shedding may also occur in asymptomatic individuals prior to the onset of symptoms. Asymptomatic transmission increases its pandemic potential several-fold (Tu et al., 2020). Indeed, COVID-19, was declared a pandemic by the World Health Organization on March 11th, 2020 because there was a dramatic and exponential increase in the number of cases and deaths associated with the disease within several months. Currently, close to the end of June 2020, there are over 10 million cases worldwide with over 500,000 deaths. Treatment options for COVID-19 are limited while several vaccines against SARS-CoV-2 are in the works. On April 29, 2020, NIH announced that

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remdesivir, an experimental drug originally developed as an RNA dependent RNA polymerase (RdRP) inhibitor against Ebola virus (EBOV), showed positive efficacy in a clinical phase 3 trial for COVID-19. Hospitalized COVID-19 patients treated with remdesivir shortened the time to recovery by 31% (from 15 days to 11 days). On May 1, 2020, the US Food and Drug Administration (FDA) granted emergency use authorization of remdesivir for treatment of COVID-19, while a formal approval is still pending.

Overview of SARS-CoV-2 genome and protein constituents

SARS-CoV, MERS-CoV and SARS-CoV-2 belong to the Betacoronavirus genus, whose genomes typically contain 5′-methylated caps at the N-terminus and a 3′-poly-A tail at the C-terminus with a highly conserved order of genes related to replication/transcription and structural components. The replication and transcription related gene is translated into two large non-structural polyproteins by two distinct but overlapping open reading frames translated by ribosomal frameshifting (Tu et al., 2020). The overlapping open reading frame, comprising two-thirds of the coronavirus genome, encodes the large replicase polyproteins 1a and 1b (pp1a and pp1b) which are cleaved by papain-like cysteine protease (PLpro) and 3C-like serine protease (3CLpro, also called Mpro). This cleavage produces 16 non-structural proteins (Nsp) including important enzymes involved in the transcription and replication of coronaviruses such as RNA-dependent RNA polymerase (RdRP), helicase (Nsp13), and exonuclease (Nsp14) (Tang et al., 2020). The 3′ one-third of the coronavirus genome is translated from subgenomic RNAs, and encodes the structural proteins spike (S), envelope (E), and membrane (M) that constitute the viral coat, and the nucleocapsid (N) protein that packages the viral genome (Tu et al., 2020). These structural proteins are essential for virus–host cell binding and virus assembly. Upon translation, the S, E, and M structural proteins are inserted into the rough endoplasmic reticulum to travel along the secretory pathway to the endoplasmic reticulum-Golgi apparatus intermediate

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compartment or coronavirus particle assembly and subsequent release from the cell via exocytosis (Tang et al., 2020).

Viral entry through the binding of SARS-CoV-2 spike proteins to ACE2 receptor

The S proteins of SARS-CoV, required for viral entry into the host target cell, are synthesized as inactive precursors and become activated only upon proteolysis (Gierer et al., 2013). The S protein has two functional domains called S1 and S2. S1 contains an N-terminal domain (NTD) and a receptor binding domain (RBD). The receptor binding motif (RBM) is located within the carboxy-terminal half of the RBD and contains residues that enable attachment of the S protein to a host cell receptor (Letko et al., 2020). The S2 subunit drives the fusion of viral and host membrane subsequent to cleavage, or 'priming,' by cellular proteases. SARS-CoV is known to gain entry into permissive host cells through interactions of the SARS-S RBD with the cell surface receptor angiotensin converting enzyme 2 (ACE2) (Wang et al., 2008). ACE2 is a negative regulator of the renin-angiotensin system and counterbalances the function of angiotensin converting enzyme (ACE), thereby maintaining blood pressure homeostasis (Kuba et al., 2005). It was shown in animal models that ACE2 promotes anti-inflammation, anti-fibrosis, and vasodilation, whereas ACE promotes pro-inflammation, fibrosis, vasoconstriction, and severe lung injury (Kuba et al., 2005). Further, through S protein binding, SARS-CoV downregulates ACE2 receptor and therefore this process not only leads to viral entry, but also potentially contributes to severe lung injury as the ACE2 pathway has protective functions in many organs. Since 83% of ACE2-expressing cells are alveolar epithelial type II cells, and these cells contain high levels of multiple viral process-related genes, including regulatory genes for viral processes, viral life cycle, viral assembly, and viral genome replication, they can facilitate coronaviral replication in the lung (Zhao et al., 2020). Sequence analysis showed that SARS-CoV-2 genome is very similar to SARS-CoV with a only a few

differences in their complement of 3' open reading frames that do not encode structural proteins (Gordon et al., 2020). Specifically, the S proteins of SARS-CoV-2 and SARS-CoV share 76.5% identity in amino acid sequences and have a high degree of homology (Xu et al., 2020b). SARS-CoV-2 also uses ACE2 as a cellular entry receptor as, in otherwise non-susceptible cells to SARS-CoV-2 infection, overexpressing human or bat ACE2 mediates SARS-CoV-2 infection and replication (Hoffmann et al., 2020a; Zhou et al., 2020). In addition, SARS-CoV-2 does not utilize other receptors such as dipeptidyl peptidase 4 (DPP4), used by MERS-CoV, or the human aminopeptidase N (hAPN) used by human CoV 229E (Zhou et al., 2020) (Ou et al., 2020). Several groups have now identified the RBD in SARS-CoV-2 and have confirmed by biochemical analyses as well as crystal structure prediction analyses that this domain binds strongly to both human and bat ACE2 receptor with a binding affinity significantly higher than that of SARS-CoV to the ACE2 receptor (Tai et al., 2020; Wan et al., 2020; Wrapp et al., 2020; Xu et al., 2020b). There has been much speculation that the high affinity binding of SARS-CoV-2 to ACE2 could mediate the increased potential for transmissibility and severity of infection. For instance, the coronavirus NL63 also uses the same ACE2 receptor for entry into the host cell as SARS-CoV, but the virus entry and outcome are vastly different, with SARS resulting in severe respiratory distress and NL63 resulting in only a mild respiratory infection (Mathewson et al., 2008). This led the authors to suggest that a lower-affinity interaction with NL63 for ACE-2 may partially explain the different pathological consequences of infection. It has been speculated that in addition to the ACE2 receptor, SARS-CoV-2 could employ other receptors for host cell entry. For example, the S protein of SARS-CoV-2 has a conserved RGD motif known to bind integrins, which is not found in other coronaviruses (Sigrist et al., 2020). This motif lies within the RBD of the S proteins of SARS-CoV-2, close to the ACE2 receptor-binding region (Sigrist et al., 2020). SARS-CoV-2 S protein can also interact with sialic acid receptors of the cells in the upper airways similar to MERS-CoV (Milanetti et al., 2020). Although the functional importance of integrins or sialic acid receptors in mediating SARS-CoV-2 S entry remains to be determined,

these may potentially increase cell tropism, viral pathogenicity, and transmission of SARS-CoV-2.

SARS-CoV-2 has multiple viral entry mechanisms

In general, coronaviruses deliver their genomes to the host cytosol by two known methods: directly fusing with the plasma membrane at the cell surface in a pH-independent manner, or utilizing the host cell's endocytic machinery in which the endocytosed virions are subjected to an activation step in the endosome. Endocytic activation is typically mediated by the acidic endosomal pH, resulting in the fusion of the viral and endosomal membranes and release of the viral genome into the cytosol (Wang et al., 2008). Fusion with the cell membrane requires that the S2 domain of the S protein be primed by cellular proteases at the S' site. SARS-CoV is known to be able to enter host cells by both directly fusing with the host membrane as well as through the endosomal pathway via cathepsin B and L (CatB/L) (Matsuyama et al., 2010). SARS-CoV can also utilize the cell surface protease transmembrane protease serine type 2 (TMPRSS2) that belongs to the type II transmembrane serine protease family. Although SARS-CoV utilizes both host cell entry pathways, it appears that the TMPRSS2 pathway is the major route of infection of SARS-CoV in the lungs. However, in the absence of TMPRSS2, SARS-CoV can also employ the endosomal late entry route for infection, as SARS-CoV viral spread is still detected in the alveoli of TMPRSS2 knockout mice (Iwata-Yoshikawa et al., 2019). Unlike other soluble serine proteases, TMPRSS2 is anchored on the plasma membrane and localized with ACE2 receptors on the surface of airway epithelial cells (Shulla et al., 2011). This colocalization makes the lungs particularly susceptible to infection. TMPRSS2 cleavage of S protein might also promote viral spread and pathogenesis by diminishing viral recognition by neutralizing antibodies. The cleavage of S protein can result in shedding of SARS S fragments that could act as antibody decoys (Glowacka et al., 2011). Although TMPRSS2

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affects the entry of virus but not the other phases of virus replication, only a small amount of S protein needs to be cleaved to enable viral or cell-cell membrane fusion, even when minute or undetectable amounts of ACE2 is available (Shulla et al., 2011). In keeping with this, the expression and distribution of TMPRSS2, but not ACE2, correlates with SARS-CoV infection in the lungs. SARS-CoV studies have shown that TMPRSS2 cleaves the S protein following receptor binding, which causes conformational changes that expose the S' cleavage site (Glowacka et al., 2011). This confers a great advantage to the viral protein by protecting the activating cleavage site from premature proteolysis and yet, ensuring that efficient cleavage occurs upon binding to the receptor on target cells (Shulla et al., 2011). Similarly, in the case of SARS-CoV-2, the host cell TMPRSS2 primes the S protein, and enhances entry and infection (Hoffmann et al., 2020a; Matsuyama et al., 2020b). SARS-CoV-2 may also utilize other host proteases such as trypsin for S protein activation (Ou et al., 2020). Similar to SARS-CoV, SARS-CoV-2 can also enter host cells through the endosomal pathway via cathepsins (Hoffmann et al., 2020a; Ou et al., 2020). Unlike SARS-CoV, the S protein of SARS-CoV-2 has a furin cleavage site at the S1/S2 boundary similar to MERS-CoV (Walls et al., 2020) (Hoffmann et al., 2020a), which likely sensitizes S proteins to the subsequent activating proteolysis occurring on susceptible target cells, facilitates virus entry and infection, and potentially increases viral transmissibility (Qing and Gallagher, 2020). Since SARS-CoV-2 can be activated by an extensive range of proteases, and given that a varied number of proteases exist on the cell surface of different cell types, SARS-CoV-2 has the capacity to infect a wide range of cells (Tang et al., 2020). Thus, it is an opportunistic virus that can utilize multiple pathways of host cell entry and infection. It is conceivable that successful treatment of COVID-19 may require a cocktail of drugs that target multiple mechanisms of action as historically seen for the treatment of HIV infection; see review (Maeda et al., 2019).

Drug development strategies

For negative-sense RNA viruses, approved therapies are currently available only for rabies virus, respiratory syncytial virus, and influenza virus (Hoenen et al., 2019). Since there are many functional similarities between SARS-CoV, SARS-CoV-2, and MERS-CoV, it is reasonable to screen drugs that were even moderately effective against SARS-CoV and MERS-CoV for SARS-CoV-2. These broad-spectrum anti-coronavirus drugs could also be used against future emerging coronavirus infections. In particular, any such drugs that have an IC_{50} in the low nanomolar range, (preferably less than 100 nM) with high efficacy in inhibiting viral infection *in vitro* would be most advantageous. Drug repurposing has been used in response to emerging infectious diseases to rapidly identify potential therapeutics. If FDA-approved drugs currently on the market for other diseases demonstrate anti-SARS-CoV-2 activity, they could be repurposed for COVID-19 treatment. Several groups have identified compounds with anti-SARS-CoV-2 activity by repurposing select FDA-approved drugs (Choy et al., 2020; Jeon et al., 2020; Wang et al., 2020b). In addition, high-throughput drug repurposing screens have also been successfully used to identify such compounds (Table 1). The National Center for Advancing Translational Sciences also provides an online open science data portal for COVID-19 drug repurposing (https://ncats.nih.gov/expertise/covid19-open-data-portal) (Brimacombe et al., 2020).

For compound screening with large scale libraries and molecular target-based assays, BSL-2 laboratories are commonly used. These assays take longer to develop but are usually without risk of infectivity to humans and are capable of higher throughput than live SARS-CoV-2 virus assays requiring BSL-3 facilities. However, the efficacy of active compounds identified from such high throughput screening (HTS) needs to be confirmed with live SARS-CoV-2 virus assays done in the BSL-3 environment. For example, virus pseudoparticles that contain viral structural proteins without the viral genome can be used to assay viral entry mechanisms. Cell

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lines expressing viral replicons that contain portions of the viral genome with reporter genes but without viral structure genes can also be used to assay viral replication mechanisms. These non-viral assays used for viral entry or replication are not infectious and can be used in a BSL-2 facility for screening large compound collections. This strategy has been used to screen compounds for BSL-3/4 viruses such as EBOV (Kouznetsova et al., 2014; Tscherne et al., 2010), Lassa virus (Cubitt et al., 2020), SARS and MERS-CoV (de Wilde et al., 2014; Dyall et al., 2014). Recently, Letko et. al. showed chimeric S proteins containing RBD of SARS-CoV-2 can confer receptor specificity to the full S protein sequence (Letko et al., 2020). This approach of non-conventional pseudotyping method is cost effective and can provide a faster way to screen viral-host interactions.

Therapeutic targets for COVID-19 can be directed towards the SARS-CoV-2 virus and its proteins or the host cell targets. Prevention of virus-host associations can fall in either of the two categories. Drugs targeting viral proteins have a major advantage as they could potentially have higher specificity against the virus while having minimal adverse effects on humans. However, drug resistance may develop rapidly after treatment, particularly in RNA viruses where mutations occur frequently. Conversely, therapeutics targeting host cells may slow the development of drug resistance as mutations in host cells are relatively rare (Hoenen et al., 2019). Importantly, drugs targeting host cells have greater potential for adverse effects. Possible treatment options under investigation for the prevention and control of SARS-CoV-2 infections in both categories are discussed below.

SARS-CoV-2 viral entry inhibitors

Antibodies

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Neutralizing antibodies can be used to prevent viral cell surface receptor binding to block viral entry. After viral entry, the viral replication cycle concludes in the assembly and budding of new viral progeny at the host-cell surface (Murin et al., 2019). These processes can be disrupted by neutralizing antibodies which bind to the viral glycoprotein to block viral egress (Murin et al., 2019). Thus, neutralizing antibodies can prevent viral entry as well as viral release, thereby blocking the infection of neighboring cells. *In vitro* neutralization assays followed by *in vivo* protection in an animal model was the standard workflow for choosing neutralizing antibodies against filoviruses such as the EBOV, which emerged as an outbreak in 2014 in West Africa (Saphire et al., 2018). A glycoprotein-targeting cocktail of antibodies rather than a single antibody design against EBOV was shown to be superior and is currently being used in areas of outbreaks (Hoenen et al., 2019). Similarly, several anti-influenza monoclonal antibodies are currently in various stages of clinical development, and most are directed towards the viral Hemagglutinin (HA) glycoprotein (Corti et al., 2017). For both EBOV and influenza, some broadly reactive antibodies lacking *in vitro* neutralizing activity have shown *in vivo* efficacy under prophylactic settings and thus, there is not a precise correlation between *in vitro* activities and *in vivo* protection (Corti et al., 2017; Tian et al., 2020). Neutralizing antibodies designed against SARS-CoV and MERS-CoV could potentially be effective against SARS-CoV-2. Several monoclonal antibodies targeting S protein of SARS-CoV and MERS-CoV have shown promising results in neutralizing infection both *in vitro* and in rodent models (Shanmugaraj et al., 2020). Since the structure of SARS-CoV-2, SARS-CoV, and MERS-CoV S protein and monoclonal antibody interaction sites have been determined, neutralizing antibodies against S protein of SARS-CoV-2 promises to be a viable therapeutic option.

Convalescent plasma therapy using plasma from recovered COVID-19 patients to treat severe cases of COVID-19 has shown positive results. Convalescent plasma contains neutralizing antibodies specifically against the SARS-CoV-2 virus, and confers passive immunity

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to the recipient, thereby improving clinical outcomes when used prophylactically and in infected patients (Casadevall and Pirofski, 2020). RBD-specific monoclonal antibodies derived from SARS-CoV-2 infected individuals was shown to have neutralizing activities against both pseudoviruses bearing the S protein as well as live SARS-CoV-2 viruses (Ju et al., 2020). Treatment with convalescent plasma was shown to be successful in a small cohort of patients (Duan et al., 2020; Shen et al., 2020). Clinical trials are currently underway to determine whether COVID-19 convalescent plasma or 'hyperimmune plasma' might be an effective treatment therapy for COVID-19. The Takeda Pharmaceutical Company has announced investigation into a new plasma derived therapy coined TAK-888 that involves removing plasma from COVID-19 survivors and extracting coronavirus-specific antibodies to stimulate a potent immune response against SARS-CoV-2 in infected patients (Barlow et al., 2020).

Proteins, peptides, small molecule compounds and drugs

Viral entry can also be blocked by proteins, peptides, or small molecule compounds that bind to the viral S protein, thereby preventing the interaction of virus and host membrane. Recombinant soluble ACE2, which lacks the membrane anchor and can circulate in small amounts in the blood, can act as a decoy to bind SARS-CoV-2 S proteins, and thus prevent viral entry (Lei et al., 2020). Clinical grade human recombinant soluble ACE2 was shown to successfully inhibit SARS-CoV-2 infection in engineered human blood vessel organoids and human kidney organoids (Monteil et al., 2020). Studies also show that soluble human ACE2 can significantly decrease SARS-CoV-2 viral entry (Ou et al., 2020) and recombinant proteins designed against the RBD of S protein of SARS-CoV-2 can successfully block entry of virus into cells (Tai et al., 2020) (Zhang et al., 2020). Another class of proteins that may be useful in blocking viral entry into cells are lectins, which bind specific carbohydrate structures but lack intrinsic enzymatic activity (Mitchell et al., 2017). Lectins may inhibit viral entry and subsequent replication by interacting with coronavirus S proteins that are heavily glycosylated (Mitchell et

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al., 2017). Griffithsin is a lectin protein isolated from marine red algae, and has proven antiviral properties (Mori et al., 2005). Griffithsin can potently inhibit viral entry by binding to the S glycoprotein and prevent SARS-CoV infection both *in vitro* and *in vivo* with minimal cytotoxic effects (O'Keefe et al., 2010). Similarly, griffithsin was shown to inhibit MERS-CoV infectivity and production *in vitro* with no significant cytotoxicity (Millet et al., 2016). Another lectin protein known as UDA was shown to significantly decrease mortality rates in a mouse model of SARS-CoV infection and was able to impede viral entry and replication (Day et al., 2009) (Kumaki et al., 2011). However, UDA requires higher concentrations than griffithsin to achieve similar inhibitions of viral infections (O'Keefe et al., 2010) and high doses of UDA has toxic effects in mice (Kumaki et al., 2011). Based on these *in vitro* as well as preclinical results, griffithsin may well prove to be an effective SARS-CoV-2 entry inhibitor.

Peptides can also be designed against the highly conserved heptad repeat region located in the S2 subunit of the S protein, which can interfere with viral and host cellular membrane fusion. A lipopeptide, EK1C4, exhibited highly potent inhibitory activity against SARS-CoV-2 S-mediated membrane fusion *in vitro* and *in vivo* (Xia et al., 2020). Small molecules that block the binding of S protein to ACE2 can also be investigated as therapeutics for COVID-19 treatment. For example, the CCR5 antagonist maraviroc, which was approved in 2007 for the treatment of HIV infections, blocks HIV from binding to its co-receptor CCR5. Thus, a specific ACE2 inhibitor may be developed that blocks the binding of SARS-CoV-2 S protein to ACE2.

Additionally, the inhibitors of host cell proteases such as TMPRSS2, furin, and cathepsin that prime viral structure proteins for membrane fusion may also prevent SARS-CoV-2 entry. Developing these types of inhibitors as therapeutics may present challenges due to differentially expressed proteases in different tissues. Therefore, developing a broad-spectrum protease inhibitor against SARS-CoV-2 might be beneficial. For example, the TMPRSS2 inhibitor

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camostat mesylate can block the entry of SARS-CoV-2 into Calu-3 human lung epithelial cells (Hoffmann et al., 2020a), but a combination of camostat mesylate and Cathepsin B/L inhibitor E-64-d is required to completely block viral entry into Caco-2 cells (Hoffmann et al., 2020a). Nafamostat is another example of a serine protease inhibitor that can inhibit SARS-CoV-2 entry and infection (Hoffmann et al., 2020b; Wang et al., 2020a). However, compared to camostat mesylate, nafamostat blocks viral entry and replication with significantly greater efficacy (Hoffmann et al., 2020b; Shrimp et al., 2020). Nafamostat, approved as a treatment for pancreatitis in Japan and Germany with no major adverse effects, may also have anti-inflammatory properties that could aid COVID-19 patients (Hoffmann et al., 2020b); clinical trials will determine its suitability as a COVID-19 therapeutic (Table 2).

Viral entry may also be inhibited by Umifenovir (also known as arbidol), which is approved for the treatment of influenza in Russia and China. Arbidol potently blocks SARS-CoV-2 entry into cells as well as inhibits post-entry stages of infection (Wang et al., 2020b). The lower EC_{50} value of 4.11 µM against SARS-CoV-2 compared to influenza viruses gives arbidol the potential to be a clinically effective therapeutic against SARS-CoV-2 (Wang et al., 2020b). One clinical trial is set to determine the effectiveness of arbidol for the treatment of COVID-19 induced pneumonia (Table 2) and several others as a combination therapy. In addition, chlorpromazine, a FDA-approved antipsychotic and clathrin-dependent endocytosis inhibitor, also has anti-SARS-CoV-2 activity *in vitro* (Plaze et al., 2020) and is currently under investigation as a potential therapeutic for COVID-19 (Table 2).

Viral replication inhibitors

Inhibitors of viral nucleic acid synthesis are the best represented class of antiviral drugs that suppress viral replication in host cells (Hoenen et al., 2019). The most successful 3CLpro

inhibitor is lopinavir, a protease inhibitor used to treat HIV infections that is usually marketed as a ritonavir-boosted form (lopinavir–ritonavir) (Zumla et al., 2016). Preliminary *in vitro* studies with ritonavir on SARS-CoV-2 infection have not shown much promise (Choy et al., 2020). However, there are clinical trials underway to test the efficacy of this drug in humans (Table 2). Specifically repurposing any inhibitors designed against SARS-CoV or MERS-CoV 3CLpro for SARS-CoV-2 may prove challenging. Although there is a high degree of sequence conservation in the active sites of SARS-CoV and MERS-CoV 3CLpro enzymes, most SARS-CoV 3CLpro inhibitors are inactive against MERS-CoV, indicating other important structural differences (Needle et al., 2015).

RdRP is another target for SARS-CoV-2 drug development. The sequence encoding the structure of RdRP in SARS-CoV, MERS, and SARS-CoV2 was found to be remarkably similar (Morse et al., 2020). Several RdRP inhibitors originally developed for other viruses are in active clinical trials to treat COVID-19 infections (Table 2). Remdesivir, a prodrug of an adenosine analog, was originally developed for the treatment of EBOV and has broad-spectrum antiviral activities against RNA viruses. Remdesivir successfully improved outcomes when used prophylactically and therapeutically in animals models of MERS (de Wit et al., 2020) as well as SARS (Sheahan et al., 2017). Treatment with remdesivir during early infection also showed significant clinical benefits in non-human primates infected with SARS-CoV-2 (Williamson et al., 2020). In COVID-19 patients, clinical improvements without adverse effects were noted when treated with remdesivir on a compassionate-use basis (Grein et al., 2020). In a randomized, controlled trial known as the Adaptive COVID-19 Treatment Trial (ACTT), COVID-19 patients that received remdesivir had significantly shorter recovery and mortality rates with no serious adverse events (Beigel et al., 2020). Due to these promising preliminary reports, remdesivir was recently granted emergency use authorization for the treatment of COVID-19 by FDA.

Other RdRP inhibitors include favipiravir and ribavirin. Favipiravir, a prodrug guanosine analog, is approved for the treatment of influenza in Japan and China. Favipiravir is not reported to have significant adverse effects, however it may increase the risk for teratogenicity and embryotoxicity (Furuta et al., 2017). Ribavirin, another guanosine analog prodrug, is used for the treatment of severe respiratory syncytial virus infection, hepatitis C viral infection, and viral hemorrhagic fevers (Tu et al., 2020; Zumla et al., 2016). However, when used as a treatment for SARS-CoV in both preclinical (Day et al., 2009) and clinical settings (Stockman et al., 2006), ribavirin did not improve outcomes but instead had adverse effects. In addition, of these three drugs, only remdesivir has shown potent inhibition of SARS-CoV-2 infection *in vitro* (Wang et al., 2020a) (Choy et al., 2020). Therefore, pursuing specific SARS-CoV-2 RdRP inhibitors is a valid approach for COVID-19 drug development, but the efficacy and toxicities of these drugs will need to be closely scrutinized.

Recently, ivermectin was shown to potently inhibit SARS-CoV-2 *in vitro* with a single treatment (Caly et al., 2020). Ivermectin is an FDA-approved drug for the treatment of parasitic infections. However, its suitability as a COVID-19 treatment is currently being examined in several clinical trials, including one that will test asymptomatic patients (ClinicalTrials.gov Identifier: NCT04407507).

Four additional enzymes specific to SARS-CoV-2 - helicase (Nsp-13), 3'-5' exonuclease (Nsp-14), Uridine-specific endoribonuclease (Nsp-15), and RNA-cap methyltransferase (Nsp-16) - may be considered as key targets for drug discovery (Gordon et al., 2020). In single stranded positive sense RNA viruses such as SARS-CoV-2, RNA helicases are essential for viral genome transcription and protein translation. Thus, inhibitors of viral helicases are attractive as therapeutic agents. At least one molecule inhibitor of SARS-CoV helicase without cell toxicity has been previously identified (Cho et al., 2015). However, despite significant efforts being made towards their development, helicase inhibitors are currently not available for clinical

use (Briguglio et al., 2011). The SARS-CoV-2 exonuclease (Nsp-14) cleaves nucleotides at 3' end of RNA strand and is required for RNA replication (Romano et al., 2020). Uridine-specific endoribonuclease (Nsp-15, EndoU) is an endoribonuclease that hydrolyzes single-stranded as well as double-stranded RNA at uridine residues. EndoU is highly conserved in all coronaviruses, which alludes to its functional importance (Hackbart et al., 2020). Although its precise role in viral pathogenesis is not well established, it likely plays a role in evading host recognition (Deng and Baker, 2018). A recent study confirmed that EndoU contributes to delayed type I interferon response by cleaving 5′-polyuridines from negative-sense viral RNAs, which otherwise activate host immune sensors (Hackbart et al., 2020). There are currently no approved inhibitors for viral specific 3'-5' exonuclease or EndoU. In coronaviruses, RNA-cap methyltransferase (Nsp-16) forms a complex with its cofactor nsp-10 (a 2-O-methyltransferase) for the addition of a cap to the 5'-end of viral RNA. This addition enables the virus to escape innate immune recognition in host cells as well as enhance viral RNA translation (Wang et al., 2015). Unfortunately, there are currently no effective inhibitors or approved drugs for these enzymes that may be used as targets for antiviral drug development.

Host cell and viral targets for antiviral drug development

Viral replication requires a number of cellular proteins and machinery. Inhibiting host cell protein function may effectively combat viral infection. These host targets include the host cell proteases TMPRSS2, furin, and cathepsin and ACE2 receptor discussed above. Additionally, the host cell autophagy pathway is used by some coronaviruses for viral replication and viral assembly. Since coronaviruses may hijack autophagy mechanisms for viral double membrane vesicle formation and replication, the inhibition of cellular autophagy may be a useful antiviral strategy (Abdoli et al., 2018; Yang and Shen, 2020). Although the drugs targeting host cell proteins are more likely to cause adverse effects, patients might tolerate a short 7- to 14-day

treatment regimen. Other complicating factors for targeting host proteins is the redundancy of human cellular function pathways and variations in different cells or tissues, which may reduce correlations between *in vitro* and *in vivo* efficacy studies. This issue could be overcome by utilizing a drug combination therapy with different mechanisms of action such as that seen with the successful treatment of HIV; see review (Maeda et al., 2019). Alternatively, a single drug with multiple activities against both viral targets and host viral replication machineries may be more effective in treating SARS-CoV-2 infection than drugs acting on one viral target. These drugs with polypharmacology against SARS-CoV-2 infection are discussed below:

Chloroquine/hydroxychloroquine (CQ/HCQ) is a well-known FDA-approved antimalarial drug that can inhibit viral infections via multiple mechanisms. The mechanism of inhibition likely involves the prevention of endocytosis or rapid elevation of the endosomal pH and abrogation of virus–endosome fusion (Devaux et al., 2020). CQ/HCQ also has anti-inflammatory properties, which has led to its clinical use in conditions such as rheumatoid arthritis, lupus, and sarcoidosis (Savarino et al., 2003). After the advent of SARS in 2003, Savarino et. al postulated that the antiviral as well as anti-inflammatory properties of CQ/HCQ might be beneficial for SARS treatment. In addition, viruses may engage host autophagic processes to enhance replication (Yang and Shen, 2020). Since CQ is a known inhibitor of autophagic flux, it may be beneficial in inhibiting viral replication. CQ was shown to be highly effective in the control of SARS-CoV-2 infection *in vitro* (Wang et al., 2020a). It can also potentially interfere with the terminal glycosylation of ACE2 receptor expression, thereby preventing SARS-CoV-2 receptor binding and subsequent spread of infection (Barlow et al., 2020). Andreani et al. found that HCQ significantly suppresses virus replication, and a combination of HCQ and azithromycin exhibits synergistic effects (Andreani et al., 2020). However, the FDA has warned that the use of CQ/HCQ, particularly when used in conjunction with azithromycin, can cause abnormal heart rhythms such as QT interval prolongation and ventricular tachycardia. As of June 15th, 2020,

FDA has revoked the emergency use authorization of these drugs for the treatment of COVID-19. Several clinical trials are underway to test the efficacy of CQ/HCQ in different settings, although a few have been withdrawn. In addition, a drug repurposing screen performed in different cell lines indicates that viral entry in lung epithelial Calu-3 cells is pH-independent (Dittmar et al., 2020). Therefore, CQ may not be effective as a treatment against SARS-CoV-2.

Emetine, approved for amoebiasis, has broad antiviral activity against Zika virus, EBOV virus, Dengue virus, human cytomegalovirus, and HIV *in vitro* and *in vivo*. Emetine may elicit cardiotoxic and myotoxic effects with high doses; however, its potency as antiviral is significantly lower than doses that cause toxicity (Yang et al., 2018). Emetine can act on multiple mechanisms such as viral RdRp inhibition, host cell lysosomal function, and blocking viral protein synthesis via inhibition of host cell 40S ribosomal protein S14 (Yang et al., 2018). A combination of 6.25 μM remdesivir and 0.195 μM emetine showed synergistic effects *in vitro* against SARS-CoV-2 infection, but some of the compounds currently undergoing clinical trials such as ribavirin and favipiravir did not show clear antiviral effects (Choy et al., 2020). This suggests that combination therapy may be a superior therapeutic option for the treatment of COVID-19 due to synergistic effects.

Niclosamide, an antiparasitic drug approved by FDA, has shown great potential for repurposing to treat a variety of viral infections including SARS-CoV and MERS-CoV by targeting both host and viral components (Xu et al., 2020a). Preliminary studies showed that niclosamide has potent antiviral activity against SARS-CoV-2 *in vitro* with and IC_{50} of 0.28 μM (Jeon et al., 2020). In addition, niclosasmide exhibits very low toxicities *in vitro* and *in vivo* (Chen et al., 2018), making it an attractive candidate.

Nitazoxanide is another antiparasitic prodrug approved by FDA with antiviral properties and is reportedly well tolerated in patients. Although the mechanisms of viral inhibition are not well understood, it is thought to target host-regulated pathways and not viral machinery

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(Rossignol, 2016). In keeping with this, it was shown that nitazoxanide significantly inhibits EBOV *in vitro* by enhancing host antiviral responses (Jasenosky et al., 2019). An *in vitro* study determined that nitazoxanide can inhibit SARS-CoV-2 at low-micromolar concentrations with a EC_{50} value of 2.12 µM (Wang et al., 2020a). Several trials are currently ongoing to determine its clinical efficacy as a treatment for COVID-19 patients or as a post exposure prophylaxis therapy.

Prophylactic treatment for SARS-CoV-2 infection

SARS-CoV-2 infects humans mainly through inhalation of virally contaminated aerosol droplets from infected subjects. Thus, nasal sprays containing agents that can neutralize the virus or block viral entry into host cells is one approach to prevent SARS-CoV-2 infection. There are preliminary reports that an antihistamine nasal spray can inhibit SARS-CoV-2 infection *in vitro* (Ferrer and Westover, 2020). In rhesus monkeys, a nasal spray formulation of IFN-alpha2b was successful in decreasing the severity of SARS-CoV viral infection (Gao et al., 2005). Meng and colleagues also reported that a nasal drop formula of recombinant human interferon alpha1b (rhIFN-α) prevented SARS-CoV-2 infection in an open label clinical trial (Meng et al., 2020). Additionally, it might be possible to achieve high local drug concentrations for drugs with low systemic distribution and/or dose limiting toxicity when delivered systemically. Therefore, nasal administration of drugs merit further studies as a useful strategy in preventing or reducing SARS-CoV-2 infection. Phytochemicals from naturally occurring plants, particularly lectins and polyphenols, might also prove to be valuable candidates as prophylactic or therapeutic treatment against SARS-CoV-2 (recently reviewed in (Mani et al., 2020)).

Other treatments in clinical trials for COVID-19

Several drugs that target viral life cycles directly as well as host biology are currently being investigated for COVID-19 and are summarized in Table 2 and depicted in Figure 1. As previously discussed, in patients with severe cases of COVID-19, excessive inflammatory responses and cytokine release likely contributes to the severity of disease stimulating lung and other systemic injuries. The early modulation of these responses may help reduce the risk of acute respiratory distress (Barlow et al., 2020). To this end, therapies such as inhibitory human monoclonal antibodies against cytokines such as interleukin-6 (IL6) are also being considered to help diminish the severity of excessive physiologic response to SARS-CoV-2. The efficacy of glucocorticoids, such as methylprednisolone or dexamethasone for the treatment of COVID-19 is yet to be determined. However, preliminary reports indicate that dexamethasone may be beneficial in critically ill COVID-19 patients (Horby et al., 2020). In addition, ciclesonide, an inhaled corticosteroid might also prove an effective therapy as it has low cytotoxicity and can potently suppress SARS-CoV-2 growth *in vitro* (Jeon et al., 2020; Matsuyama et al., 2020a).

Vaccines for SARS-CoV-2

Treating COVID-19 with drugs or convalescent plasma does not confer immunity, hence there remains an unmet need for immediate and long-term disease prevention in the form of a vaccine. According to WHO, there are over 100 SARS-CoV-2 vaccine candidates under preclinical development comprising of: 1) DNA-based, which contains DNA encoding immunogen in plasmids 2) inactivated-whole virus, which are heat or chemically inactivated 3) live-attenuated, which contain viable but weakened virus 4) RNA-based, where an RNA encoding the immunogen is directly introduced into the host 5) Replicating and non-replicating viral vector-based, where viral vectors are used to introduce DNA-encoding immunogenic into the host 6) protein subunits, which contains portions of a pathogen 7) virus-like particle-based vaccines, which contain non-pathogenic virus-like nano particles similar in composition to the

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virus of interest (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). Several vaccines from these categories are also under clinical investigation. The types of vaccines and their platforms used are summarized in Table 3. The advantages and disadvantages of using different platforms of vaccines vary, and are reviewed elsewhere (Amanat and Krammer, 2020).

There are high expectations for these vaccines to be available for distribution within a year. Currently, there is published data from only one study led by. Undoubtedly, an effective vaccine is the ultimate tool for COVID-19 disease prevention, but there are some important aspects to consider. SARS-CoV and MERS-CoV emerged almost 20 and 10 years ago, respectively. To this date, there are no approved vaccinations to prevent either of these diseases, although there are several candidates in the unlicensed preclinical stage. Effective vaccines are still not available for many infectious diseases such as malaria, HIV, EBOV and Zika virus. Vaccine development typically takes 10 to 15 years and is associated with high costs (Zheng et al., 2018). In addition, rapid mutations arising in viral RNA could potentially render these vaccines ineffective. RNA viruses are known to mutate with high frequency, but thus far there does not seem to be many differences in the S protein amino acid residue sequences in emergent SARS-CoV-2 variants from different countries (Robson, 2020). Antibodies induced by vaccination could also potentially increase the risk and severity of disease in subsequent host–pathogen encounters (Kulkarni, 2020). The production of these antibodies may sometimes prove beneficial to the virus instead of the host by facilitating viral entry and replication in the target cell in a phenomenon known as antibody-dependent enhancement (ADE) of infection (Kulkarni, 2020). ADE has been noted in cases of dengue virus, HIV, respiratory syncytial virus and influenza virus, but has not been confirmed for SARS or EBOV (Kulkarni, 2020). However, preliminary results in animal models of SARS-CoV-2 infection are promising. In both rodents (Corbett et al., 2020) as well as non-human primates (Gao et al., 2020), vaccinations against

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SARS-CoV-2 have shown protection without any observable ADE. In addition, positive outcomes have been noted in participants of small study in terms of tolerability and immunogenicity (Zhu et al., 2020).

Perspectives and Conclusion

For safe and effective viral therapeutics or vaccine development, it necessitates that data initially be obtained from preclinical *in vitro* or animal models. However, translation of preclinically developed drug candidates are often not reproducible in human clinical trials (Seyhan, 2019). For example, remdesivir efficiently suppressed EBOV replication *in vitro* with nanomolar activity and also protected 100% of infected animals from mortality (Warren et al., 2016). However, these results were not recapitulated in humans for the treatment of EBOV infections and the remdesivir treatment group was terminated due to low efficacy and increased toxicities (Mulangu et al., 2019). In general, viral infections are highly dependent on host cells. Therefore, successful clinical translation of SARS-CoV-2 drugs would require careful considerations of the testing platform. These might include suitable cell models for *in vitro* viral infection assays as well as human induced pluripotent stem cell derived airway and gut organoids.

SARS-CoV infection was curtailed by rigorous isolation, but MERS-CoV infection is still a concern, albeit at lower infectious rate than SARS-CoV-2. SARS-CoV-2 infections present a clinical challenge as it is highly transmissible in part due to asymptomatic transmission. Therefore, the approach taken with SARS-CoV would certainly help curtail the spread of SARS-CoV-2, but with the current spread of disease, such large-scale isolation and quarantine efforts have proven difficult. Therefore, better therapeutic options are essential. Multi-target treatment approaches of drug combination therapy have been successful with HIV treatment and will likely

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be a viable therapeutic strategy for the treatment of COVID-19. Combination drug therapy has been extensively used for the treatment of HIV, cancer, as well as severe bacterial and fungal infections. Recently, COVID-19 patients treated with the triple antiviral drug cocktail lopinavir-ritonavir-ribavirin with interferon beta-1b compared to lopinavir-ritonavir alone showed significant improvements in clinical outcomes (Hung et al., Lancet 2020). In addition, the combination drugs elicited no further side effects compared to the two-drug controls. Combination drug therapy may increase toxicity compared to single drug treatment. A meta-analysis of published trials evaluated the efficacy and toxicity of two-drug full dose combination therapy versus a single full dose drug for the treatment of rheumatoid arthritis (Felson et al., 1994). The study concluded that combination therapy led to a 9% patient withdrawal rate due to significant increases in adverse effects. Therefore, with combination therapy approaches, drugs with dose limiting toxicity as monotherapies can be utilized with lower individual drug doses, thereby reducing toxicity and synergistically enhancing therapeutic efficacy (Hoenen et al., 2019; Sun et al., 2016). As an example, several FDA-approved drugs found in a drug repurposing screen showed activity against EBOV, but were not clinically useful as their plasma concentrations were not high enough to inhibit infection in humans (Kouznetsova et al., 2014). However, drug combination therapy using three of these drugs at low concentrations were able to effectively block EBOV infection *in vitro* (Sun et al., 2017). Thus, synergistic drug combinations can be particularly useful for drug repurposing (Zheng et al., 2018). For COVID-19, drug combination therapies with multiple agents that have different mechanisms of action including inhibition of viral entry and replication, as well as inhibition of host immune responses would be a practical and useful approach for disease intervention.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Shyr, Gorshkov, Chen, Zheng

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Footnotes

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Figure Legends

Figure 1. SARS-CoV- 2 spike (S) protein binds the cell surface receptor ACE2 on host cells. Viral genome is delivered into the host cytosol by: 1) directly fusing with the plasma membrane after being cleaved and activated by the serine protease TMPRSS2 or 2) using the host cell's endocytic machinery in which the endocytosed virions are subjected to an activation step in the endosome. The viral genome also functions as the messenger RNA, which is translated into proteins such as 3CLPro, PLpro and RdRp, by host cell machineries. SARS-CoV-2 genome also encodes the structural proteins (S), envelope (E), membrane (M), nucleocapsid (N). RdRP is essential for viral replication and therefore is an attractive target for anti-SARS-CoV-2 drugs. Drugs that are currently in clinical trials are shown here in red, along with their targets of viral life cycle or viral-host interactions.

Table 1. High-throughput drug repurposing screens against SARS-CoV-2

Cell line	Assay type	Strain of SARS-CoV-2	Library screened	Reference
Caco-2	CPE	Unspecified	5632 compounds including 3488 compounds which have undergone clinical investigations[a]	(Ellinger et al., 2020)
Vero E6	Primary screen: CPE Follow up: N protein immuno-fluorescence	HKU-001a in 1' screen USA-WA1/2020 for follow up	LOPAC 1280 and ReFRAME[a] library	(Riva et al., 2020)
VeroE6	CPE	BavPat1 strain	Prestwick Chemical Library (1,520 approved drugs)	(Touret et al., 2020)

[a]*Proprietary library*

Table 2: Clinical therapies for COVID-19

Compound/treatment	Target	Phase	ClinicalTrials.gov Identifier	Approved for other clinical treatment	Status
Camostat Mesilate	Serine proteases, ex. TMPRSS2	Phase 1/2	NCT04321096	Acute pancreatitis (Japan)	Ongoing
Chlorpromazine	Clathrin-mediated endocytosis	Phase ½ Phase 3	NCT04354805 NCT04366739	Schizophrenia, manic-depression, nausea, anxiety	Not yet recruiting Not yet recruiting
Ciclesonide, an inhaled corticosteroid	Viral nonstructural protein 15 encoding an endonuclease and host process	Phase 2	NCT04330586	Asthma and allergic rhinitis (Schaffner and Skoner, 2009)	Not yet recruiting
Favipiravir (Avigan) with Tocilizumab	RdRp IL-6	Not Applicable	NCT04310228	Influenza (Japan)	Ongoing
Hydroxychloroquine and chloroquine	Antiviral properties unclear	Various	Multiple	Malaria, Autoimmune diseases (ex. lupus, rheumatoid arthritis)	Varies
Interferon Alpha-1b nasal drops	Host immune response to virus	Phase 3	NCT04320238	None	Ongoing
Ivermectin	Viral transport into host nucleus	Varies	Multiple	Antiparasitic	Ongoing
Lopinavir + Ritonavir (Kaletra)	3CLpro	Phase 4	NCT04252885	HIV	Ongoing; preliminary results show no benefit beyond standard care (Baden and Rubin, 2020).

Lopinavir-Ritonavir + Ribavirin and Interferon beta-1b	3CLpro, Viral polymerase, Host immune response to virus	Phase 2	NCT04276688	HIV	Completed; significant improvement in outcomes (Hung et al., 2020)
Nafamostat	Serine protease	Phase 1	NCT04352400	Pancreatitis (Japan and Germany)	Not yet recruiting
Niclosamide	Viral and host processes	Phase 2 and 3	NCT04345419	Anthelminthic drug	Not yet recruiting
Nitazoxanide	Viral and host processes	Various	Multiple	Antiparasitic drug	Ongoing
Remdesivir	RdRp	Phase 3	NCT04257656 NCT04252664 NCT04292899 NCT04280705	HIV	Terminated Terminated Ongoing Ongoing
Tocilizumab or Sarilumaub	human monoclonal antibody (mAb) that inhibits the interleukin-6 (IL-6) pathway by binding and blocking the IL-6 receptor	Various	Multiple	Multiple, including chimeric antigen receptor T cell-induced cytokine release syndrome, other autoimmune conditions (Barlow et al., 2020)	Ongoing
Umifenovir (Arbidol)	Viral membrane fusion of influenza a and b	Phase 4	NCT04260594	Influenza (Russia and China)	Not yet recruiting

Table 3: Potential vaccines in clinical trials for COVID-19 as of June 29th, 2020

Vaccine category	Vaccine type	Vaccine developer	Phase	Vaccine identifier
DNA based	DNA plasmid + electroporation	Inovio Pharmaceuticals	Phase 1	NCT04336410
	DNA vaccine, GX-19	Genexine Consortium	Phase 1	NCT04445389
Inactivated virus	Inactivated	Beijing Institute of Biological Products	Phase 1/2	ChiCTR2000032459
	Inactivated	Wuhan Institute of Biological Products	Phase 1/2	ChiCTR2000031809
	Inactivated	Sinovac Research & Development Co., Ltd	Phase 1/2 Phase 1/2	NCT04352608 NCT04383574
	Inactivated	Chinese Academy of Medical Sciences	Phase 1/2	NCT04412538
Nonreplicating viral vector	Adenovirus type 5	CanSino Biological Inc; preliminary results (Zhu et al., 2020)	Phase 1	NCT04313127
		Beijing Institute of Biotechnology	Phase 2	ChiCTR2000031781
	ChAdOx1-S	University of Oxford/AstraZeneca	Phase 3 Phase 2/3 Phase 2/3 Phase 1/2 Phase 1/2	ISRCTN89951424 2020-001228-32 NCT04400838 2020-001072-15 NCT04324606
	Adenoviral	Gamaleya Research Institute	Phase 1 Phase 1/2	NCT04436471 NCT04437875
	Adeno based	Chinese Academy of Medical Sciences	Phase 2	NCT04341389 NCT04412538
Protein subunit	Recombinant SARS-CoV-2 trimeric S protein subunit	Clover Biopharmaceuticals Inc./GSK/Dynavax	Phase 1	NCT04405908
	Recombinant protein (RBD Dimer)	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd/ The Second Affiliated Hospital of Chongqing Medical University/Beijing Chao Yang Hospital	Phase 1	NCT04445194

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	Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Novavax	Phase 1/2	NCT04368988
RNA	Messenger RNA in lipid nanoparticle	Moderna/NIH/NIAID; related preclinical study (Corbett et al., 2020)	Phase 1 Phase 2	NCT04283461 NCT04405076
	Messenger RNA in lipid nanoparticle	BioNTech / Fosun Pharma / Pfizer	Phase 1/2 Phase 1	2020-001038-36 NCT04368728
	mRNA	Curevac	Phase 1	NCT04449276
	mRNA	People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech.	Phase 1	ChiCTR2000034112
	Self-amplifying RNA in lipid nanoparticle	Imperial College London	Phase 1	ISRCTN17072692

Source: who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines; clinicaltrials.gov; clinicaltrialsregister.eu

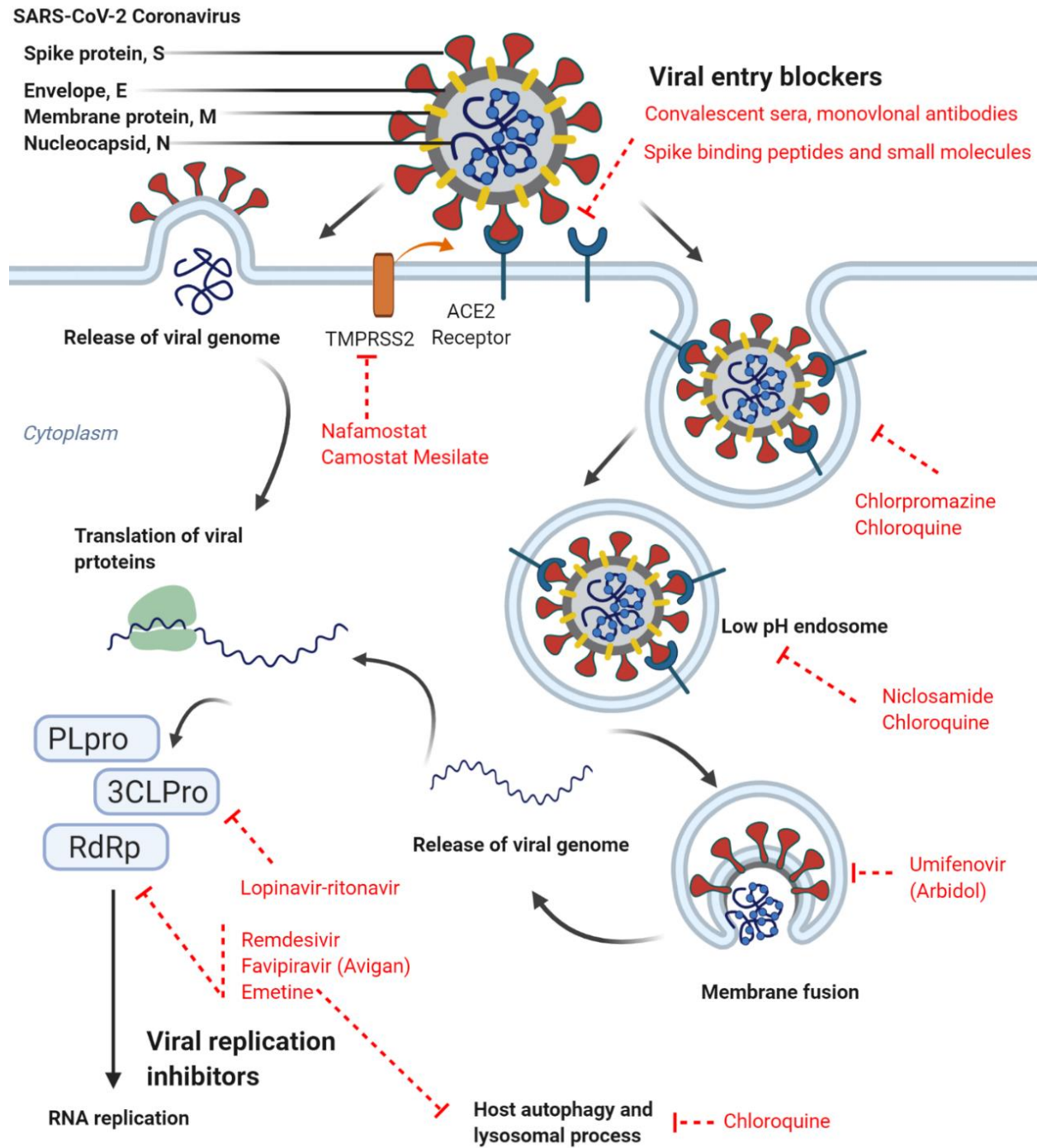Figure 1. Potential therapeutic targets against SARS-CoV-2 currently in clinical trials.

Figure created in BioRender.