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Perspective

Systems and Clinical Pharmacology of COVID-19 Therapeutic Candidates: A Clinical and Translational Medicine Perspective

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A B S T R A C T

Over 50 million people have been infected with the SARS-CoV-2 virus, while around 1 million have died due to COVID-19 disease progression. COVID-19 presents flu-like symptoms that can escalate, in about 7−10 days from onset, into a cytokine storm causing respiratory failure and death. Although social distancing reduces transmissibility, COVID-19 vaccines and therapeutics are essential to regain socio-economic normalcy. Even if effective and safe vaccines are found, pharmacological interventions are still needed to limit disease severity and mortality. Integrating current knowledge and drug candidates (approved drugs for repositioning among >35 candidates) undergoing clinical studies (>3000 registered in *ClinicalTrials.gov*), we employed Systems Pharmacology approaches to project how antivirals and immunoregulatory agents could be optimally evaluated for use. Antivirals are likely to be effective only at the early stage of infection, soon after exposure and before hospitalization, while immunomodulatory agents should be effective in the later-stage cytokine storm. As current antiviral candidates are administered in hospitals over 5−7 days, a long-acting combination that targets multiple SARS-CoV-2 lifecycle steps may provide a long-lasting, single-dose treatment in outpatient settings. Long-acting therapeutics may still be needed even when vaccines become available as vaccines are likely to be approved based on a 50% efficacy target.

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Introduction

The COVID-19 epidemic caused by the SARS-CoV-2 virus has reached pandemic proportions and caused worldwide instability. With limited ICU beds and insufficient respiratory support, healthcare systems were unprepared to treat patients who progressed to a severe acute respiratory syndrome, the dire form of the COVID-19 disease. The scientific community is still learning about viral mechanisms, infection, and immune response time-courses, especially in high-risk subpopulations with pre-existing health conditions where the infection has demonstrated a higher likelihood to degenerate into a critical condition (e.g., elderly[1]). An effective and safe vaccine has the theoretical potential to rectify an epidemic. At present, there is no approved vaccine against SARS-CoV-2. While there are more than 100 COVID-19 vaccine projects in various stages of development, it will take time to validate a quality product that is effective, safe, and scalable. Even though the preliminary data on some vaccine candidates seem promising in providing a high level of protection, their durability and long-term safety remain to be evaluated over time. The findings of these early studies will probably result in improvements and identification of required epitopes and antigens that will elicit a protective immune response. This will necessarily further extend development times. Thus, it is critical to find a set of effective pharmacological strategies that can prevent viral infection, curb transmissibility, and lower the risk for disease progression. This manuscript discusses therapeutic strategies from two perspectives: (I) Agents intended to inhibit

viral replication (antiviral strategy); (II) Agents intended to modulate host immune/inflammatory overreactions (immunoregulatory strategy). These two approaches may be deployed in sequence or over the course of a viral infection and disease development. First, the antiviral strategy is intended to lower the risk of disease progression and severity, which may lead to death. Second, if the antiviral time window (early phase) is missed, an immunoregulatory strategy may be considered to reduce the deleterious effects of a hyperimmune response (later phase).

Current antiviral drug candidates tested or approved for COVID-19 are compounds that have been repositioned from other indications. Generally, drugs can be repositioned for the treatment of the SARS-CoV-2 infection if they have evidence of in vitro inhibition against human coronaviruses (CoV). For example, *remdesivir*, which was originally intended for treating Ebola, has been recently approved for the treatment of COVID-19 requiring hospitalization; the anti-malaria drug *chloroquine* is also being evaluated for COVID-19 treatment; the anti-HIV drug combination *lopinavir-ritonavir* is used off-label in many lower-income countries against COVID-19. Antivirals, however, may fall short in severely ill COVID-19 patients. At later stages of viral infection or when subjects exhibit severe COVID-19 symptoms, immunoregulatory agents such as *dexamethasone* may be a more appropriate agent to ameliorate severe and critical conditions. As of today, there are more than three thousand clinical trials registered in *clinicaltrials.gov* with more than 30 FDA-approved drugs repositioned for COVID-19 alone or in combination with/without supportive care (e.g., oxygen therapy). Although many questions remain, a unified and integrated pharmacological strategy against COVID-19 may enable informed decisions on prioritizing the optimal use of clinical testing and global resources.

Questions regarding therapeutic windows, regimens, and effectiveness in drug repositioning, as well as the dissemination and cost-effectiveness of therapy, are issues that, in times of pandemic, call for readiness and low cost. Systems Pharmacology is a novel interdisciplinary concept envisioned by stakeholders interested in integrating real-world data produced in the laboratory and the marketplace into a novel quantitative tool. This is based on computer modeling and simulation, plus the use of artificial intelligence, where possible, with the scope to de-risk drug development and clinical pharmacology.[2] Systems Pharmacology has the potential to combine evolving COVID-19 knowledge by relating pathology, viral load time-course, and pharmacological strategy into an optimized therapeutic and drug development methodology. For instance, *remdesivir* pediatric dose included in the label (hospitalized weighing 3.5–40 kg, or <12 of age weighing >3.5 kg) was inferred from adults by the use of the computational physiologically based pharmacokinetic modeling (PBPK).[3] In order to apply Systems Pharmacology to rapidly repositioning and deploying new strategies against COVID-19, we need collective and quantitative knowledge of the infection, the disease, and the pharmacological landscape. In this text, we start with a general quantitative overview of SARS-CoV-2, COVID-19, mechanisms of infection, viral load dynamics, and pharmacology, all together to form a timeline of infection with the scope of timing the best intervention. We then introduce opportunities and applications for Quantitative Systems Pharmacology (QSP) for designing COVID-19 therapies, such as the deployment of physiologically-based pharmacokinetic modeling (PBPK) for drug repurposing. We conclude the article proposing proof-of-concept technology, particularly, introducing in the COVID-19 landscape, the idea of transforming short-acting antivirals into long-lasting drugs for the COVID-19 long-acting therapy. Modeling utilization will reveal valuable information for drug development and clinical pharmacology with the potential to change the course of current and future pandemics.

COVID-19 Disease Overview

Epidemiology

Since the Fall 2019 outbreak in Wuhan, China, about 50 million cases of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection have been reported worldwide.[4] So far, about 35 M people have recovered and 1 M have died from Coronavirus Disease 2019 (COVID-19), the disease caused by the SARS-CoV-2 infection.[4] In August 2020, it was documented that new cases amounted to 0.21 M/day with 0.005 M/day deaths.[4] The death toll has surpassed the H1N1 "swine" flu virus pandemic reported 10 years ago by which about 0.3–0.5 M people worldwide died.[5]

SARS-CoV-2 is a novel single-stranded RNA beta-coronavirus. SARS-CoV-2 presents epidemiologically with low traceability and a high reproductive number, R_0. Thus far, collating the available reports for R_0, we estimate R_0 to be in the range of 2.8–5.7. For comparison, R_0 is 1.5 for influenza H1N1 and 11 for highly transmissible varicella-zoster.[6,7] The fatality rate for SARS-CoV-2 infection varies from 7.2% in Italy to 0.7% in South Korea with the latter being a more accurate representation of the SARS-CoV-2's fatality rate as South Korea has carried out more extensive population-wide viral screenings.[4] Additionally, fatality rates may be inflated due to confounding factors from preexisting co-morbidities or co-infections such as cardiovascular disease, diabetes, chronic respiratory disease, and cancer.[8–10]

Clinical Symptoms

Mild symptoms should appear around 5 days after the putative event of infection. These symptoms include fever (80%), chills (15%), sputum (32%), cough (55%), rhinorrhea (19%), sore throat (23%), malaise (18%), myalgia (18%), and diarrhea (18%).[11] Hence, COVID-19 can initially present with symptoms similar to that of seasonal influenza. So, in some people, COVID-19 resolves via the standard of care for influenza. However, in a fraction of symptomatic patients (especially in subpopulations at risk), mild symptoms can deteriorate into severe clinical signs typical of a severe respiratory syndrome, which could lead to death by lung or another organ failure. A thorough report from the Chinese Center for Disease Control and Prevention shows that among 44,672 patients with COVID-19, 81% of cases were classified as "mild".[11] Of the mild-displaying symptoms, 19% progressed to more severe symptoms (14% as severe; 5% as critical), while 81% recovered with standard care.[11] There is a blurred line between "severe" and "critical" patients: in general, severe individuals display clear pneumonia symptoms that might or might not require respiratory support; critically ill patients need to be admitted to intensive care units (ICU) in the hospital and put on respiratory aid (e.g., physical positioning, oxygen supplementation or positive-pressure ventilation) to provide sufficient oxygenation to sustain life. Among critically ill patients, 49% might die.[11]

Mechanisms of Infection

Based on current virology, viral host interactions, and the time-course of disease development, we have provided an integrated physiological, cellular, and intracellular schematic of proposed mechanisms leading to disease development and host immune response. The SARS-CoV-2 virus's most likely entry point is through exposure to aerosolized viral particles in the air that subsequently infect the human airway's epithelium (Fig. 1a). SARS-CoV-2 features a long genetic sequence located on a positive-sense strand coupled with nuclear proteins N. The genetic material is enclosed in a typical coronavirus structure made of spike proteins S, membrane proteins M, and envelope proteins E (Fig. 1a).
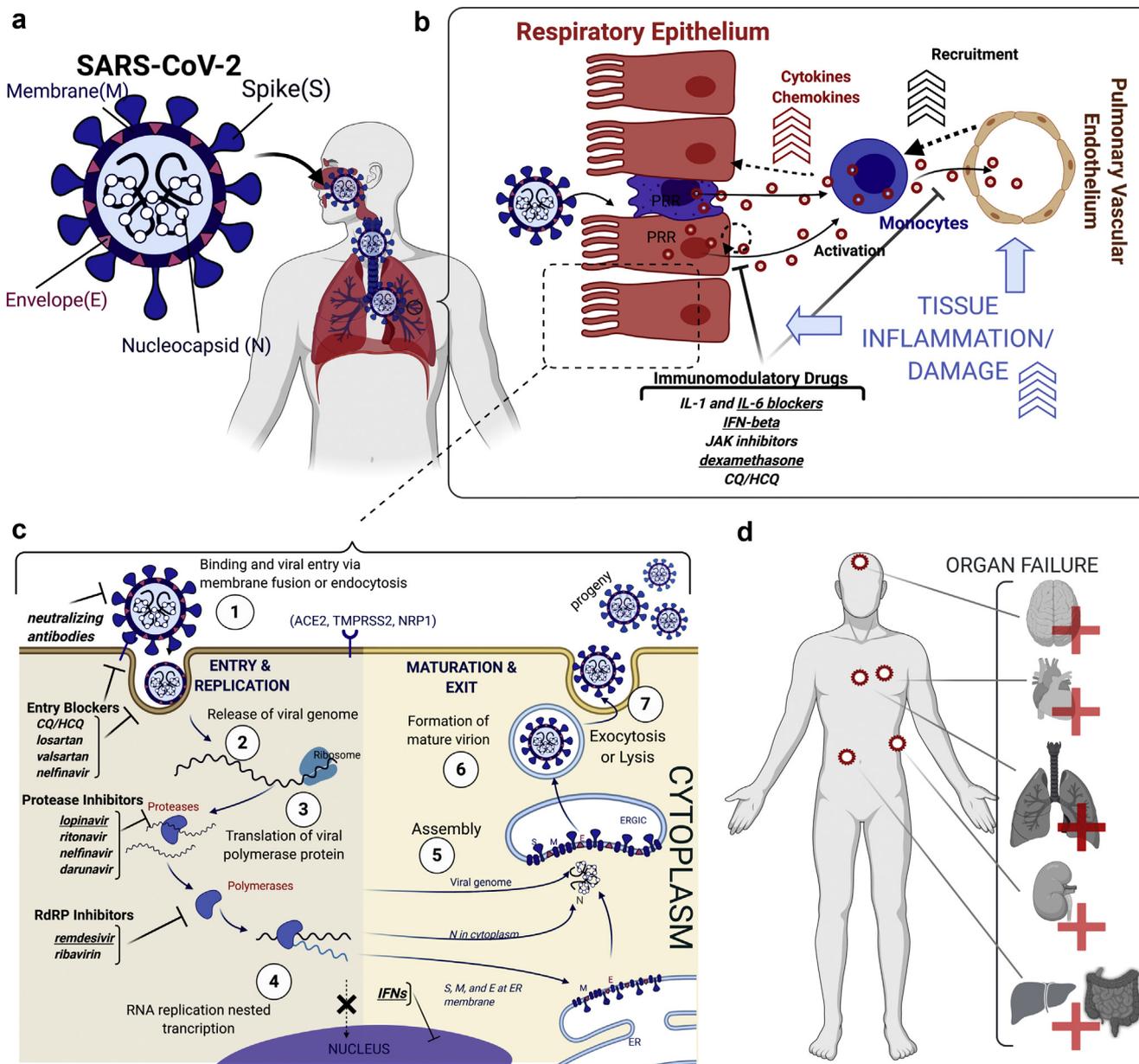
Fig. 1. Schematic representation about SARS-CoV-2: (a) viral structure and mode of infection, (b) immune response and proinflammatory pathways, (c) replication cycle in host cells leading to (d) multiple organ failure in late-stage infection. Panel a: The structure of the SARS-CoV-2 virus consists of a membrane made up of membrane proteins M, envelope E, and protruding spike proteins S. Inside the viral particle the long viral single-strain RNA is associated with nucleocapsids N. Upper airways are exposed to viral particles, which subsequently invade the respiratory epithelium in both the upper and lower respiratory tracts. The virus attacks the host by attaching via the viral spike glycoprotein (S) to bind to host membrane receptors ACE2. Panel b: The lung epithelium and resident monocytes trigger an innate immune response when viral RNA activates cellular pattern recognition receptors (PRR). This causes the secretion of several different cytokines and chemokines such as interferons (IFN) and interleukins (IL) to induce an immune response. Dysregulation of proinflammatory processes by the virus can lead to a cytokine storm where hyper-inflammation could lead to severe tissue damage and cell lysis. Pharmacological opportunities to normalize the immune response causing the cytokine storm are under evaluation, including IL-blockers and corticosteroids. Panel c: Viral replication occurs mostly in the cytoplasm of lung epithelia. The virus initially infects cells by entering via membrane fusion or endocytosis. The viral genome is released and undergoes translation processes to produce viral structural and non-structural proteins. Important viral proteins in the lifecycle are proteases and polymerases. These proteins allow for viral replication to develop mature virions. The virions exit the host cell by exocytosis and cellular lysis. Progeny is released to infect other cells. Several existing FDA-approved antiviral drugs are repositioned to inhibit different steps of the lifecycle, from entry/fusion (e.g., *hydroxychloroquine*) to inhibiting proteases (e.g., *lopinavir*), or inhibiting the polymerase (e.g., *remdesivir*). Interferons (IFN) and chloroquine may have double antiviral and immunomodulatory effects. Panel d: In addition to local lung damage, in advanced patients, other distant organs such as the kidney, liver, and gastrointestinal tract may suffer from failure or exacerbate preexisting conditions to failure.

Viral particles gain access to the cells lining the respiratory epithelium and the infected cells generate new viruses that can spread through cell-to-cell or cell-free interactions. As a response, the host infected cell flags the infection to the immune system upon lysis or shedding of the virus to nearby cells and resident monocytes (Fig. 1b). The resident monocytes, as well as the epithelium cells, release inflammatory factors or signals as cytokine and chemokines, which recruit blood and lymph node mononuclear cells and trigger other pro-inflammatory mechanisms. In some individuals, dysregulated signaling could set off the so-called "cytokine storm", the most dangerous consequence associated with COVID-19 disease progression (for more on the cytokine storm, see Mechanisms of a Cytokine Storm).

While the body arranges for self-defense, viral replication carries on in the lung cell cytoplasm (Fig. 1c). These lung epithelial cells, especially those in lung alveoli (air sacs), are critical to the exchange of CO_2/O_2 in the blood. This is why a significant fraction of infected patients may experience large drops in blood oxygen levels. Once cells become infected or lysed, they are no longer able to oxygenate the blood. At the cellular level, the viral infection should commence with the attachment of viral spikes to the host cell receptors ACE2, primed by TMPRSS2. While the exact multi-meric structure and conformation change requirement in host receptors for viral entry is under debate, once inside, the invading virus releases genetic material and accessory proteins into the host cytoplasm. SARS-CoV-2's RNA functions as an mRNA directing the synthesis of polyproteins and two proteases (M^{pro} and PL^{pro}). Proteases play an essential role in cutting polyproteins into functional pieces, i.e., the structural proteins (*M, S, E, N,* Fig. 1a), and non-structural proteins (NSP). Among NSPs, NSP12 is the RNA-dependent RNA-Polymerase (RdRP) operating as the viral replication enzyme (See[12] for the full list of NSP with function). Newly created *S, M,* and *E* proteins fuse with the membrane of an ER-Golgi intermediate compartment yielding a new virion capsule. *N* proteins attach to the replicated viral genome and are incorporated in the newly formed virion. The virions are believed to exit the cell via both exocytosis and cell lysis, perhaps depending on the viral load achieved and host response efficiency, as the latter is a more destructive mechanism. The progeny released are ready to infect other cells repeating the abovementioned cycle at an exponential pace.

In severe conditions, patients require oxygenation support to lower the risk of respiratory failure. Other failures can occur in distant organs, such as the heart, brain, GI, and kidney. Any of these failures can be fatal (Fig. 1d, see later the hypothesized mechanisms causing organ failure during a cytokine storm, Mechanisms of a Cytokine Storm).

Mechanisms of a Cytokine Storm

SARS-CoV-2 is a pathogen that is sensed by the innate immune system in humans. Although the SARS-CoV-2 immune response is still elusive, we rely on the joint understanding of current and past epidemic causing CoVs (e.g., 2003 SARS-CoV-1 and 2012 MERS-CoV). Viral RNA is believed to be recognized by pattern recognition receptors (PRR) in humans. Upon PRR activation, downstream signaling cascades trigger the secretion of cytokines. Another mechanism for immune/inflammatory activation could involve the release of destructive enzymes from lysed cell's lysosomes. Among cytokines, type I/III interferons (IFN) are considered the most important for antiviral defense, but other cytokines, such as interleukins (IL-1 or IL-6), are also released in response to CoV viral infection. In concert, IL and IFN may induce antiviral activities to clear virally infected cells, and later on, potentiate the adaptive immune response. However, CoVs have developed mechanisms to evade innate responses.[13]

Among SARS-CoV-2 evading markers, patients with severe COVID-19 demonstrate remarkably impaired type I IFN (IFN-alfa and IFN-beta) signatures as compared to mild or moderate cases, hence suggesting viral inhibition of IFN induction and signaling.[14] It is likely that to prevent the downstream signaling of IFN release, CoV proteins inhibit the steps bridging the receptor IFNAR2 and transcription STAT1 (JAK pathway) resulting in poor type I IFN production. Furthermore, viral NSP9 and NSP10 could induce, among others, IL-6 over-production, potentially by inhibiting the *NKRF* gene (an endogenous NF-kB repressor).[13] Collectively, cytokines from infected lung cells and those released from monocytes elicit inflammatory pathways in resident and circulating monocytes. In some patients, the dysregulation of these proinflammatory processes by the SARS-CoV-2 infection leads to a cytokine disease, also known as a "storm".

A cytokine storm causes lung hyper-inflammation and nonspecific attacks to the host, which could lead to tissue damage, like those observed in COVID-19 patients' lungs. Lung tissue damage produces particulate matter in the bloodstream (dead cells plus immune precipitate), which may lead to the formation of thrombi or blood clots. These blood clots may be the cause of distant organ failures, such as brain infarction and heart attacks. Other organs could also fail, such as the kidney, liver, and gastrointestinal tract. This hypothesis is consistent with the finding that alveolar-capillary microthrombi incidence is 9 times more prevalent in COVID-19 patients compared to those with influenza.[15]

Infection Timeline: Pathology, Viral Load, Serology, and Pharmacology

Based on current knowledge, as presented above, about the virus and host immune response, we provide a quantitative link between the symptoms, pathology, infection mechanisms, and pharmacological actions starting from the time of infection. We have extracted information from COVID-19 viral dynamics, host response, and disease progression, which are graphically summarized in Fig. 2. The timeline schematic in Fig. 2 is an integrated understanding based on peer-reviewed publications and verified COVID-19 consortia databases employing text analytics and network meta-analysis (implemented by AI groups through open-source web tools).[16-18] However, it should be noted that correlation analysis of the viral load with disease progression is still limited by data quality, which is thwarted by several factors, such as the sampling site, timing, and assay precision of the test.

As shown in Fig. 2, it is likely that, soon after infection, viral load increases rapidly starting from day 2 in the upper respiratory tract. It has been shown that ACE2 is expressed with higher cell density in the upper respiratory tract which highlighted the nasal susceptibility to SARS-CoV-2 with likely subsequent aspiration-mediated virus seeding to the lung in SARS-CoV-2 pathogenesis.[19] This would seem to signify a higher probability of initial infection in the upper airways. This is why viruses found in the nasal mucosal droplets are the likely source of transmission to people in close contact. It is unclear if the lower respiratory tract follows suit or has a delay. It is also unclear what the intensity of the viral load is in the lower tract. Recent findings in patients with critical conditions suggest that lower tract delay is minimal while the magnitude is much higher than in the nasal tract or throat. Furthermore, the lower tract viral load seems to linger much longer, even if the upper tract samples are tested negative in the same individuals.[20] Regardless, symptom onset is expected around day 5 from infection. The viral load in the upper tract should peak around day 10 from infection.[21-24] Therefore, with the viral load depicted in Fig. 2, pre-symptomatic subjects can transmit the virus for 2−4 days before testing. Another problem in this pandemic is that asymptomatic individuals (i.e., not exhibiting symptoms) can still transmit the virus as long as their viral load is high enough. According to epidemiological modeling, 43% of exposed individuals to SARS-CoV-2 infection remain asymptomatic, further hindering traceability and intervention capacity.[16]

The turning point of the disease may begin around day 15 from infection, where symptoms either begin to resolve or further deteriorate into severe respiratory conditions. Seroconversion (antibodies against SARS-CoV-2) in uncomplicated patients may start soon after the viral load peak[25]; however, antibody appearance in the blood is generally observed around day 21[26]. Initially, SARS-CoV-2 specific antibodies are detected as an IgM class of
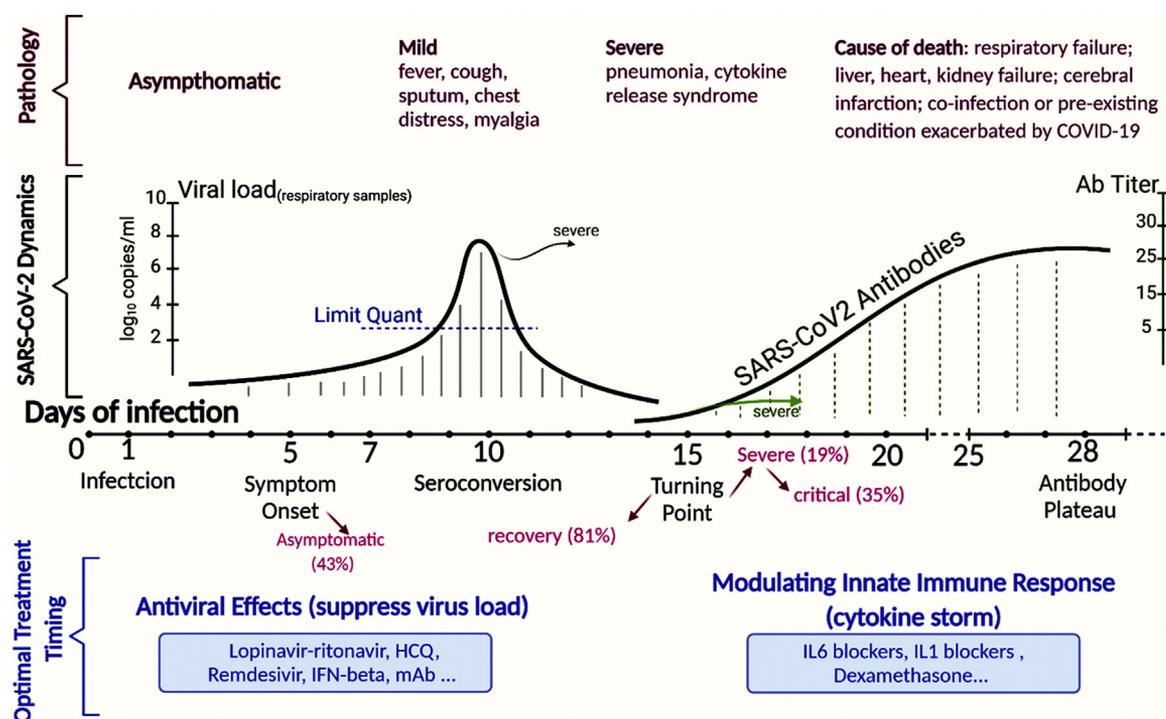
Fig. 2. Time-course of SARS-CoV-2 infection with viral load, pathology, host response, and ensuing potential for seroconversion. Infection timeline based on data collected via AI-based searches on the most relevant/impactful literature (as of September 2020). Upper panel (Pathology): After a SARS-CoV-2 infective exposure, 57% of the subjects exhibit COVID-19 mild symptoms on day 5 (dry cough, fever, myalgia, etc.), while the remainder 43% remain asymptomatic (yet contagious). Of those displaying symptoms, 19% may progress to severity (pneumonia, cytokine storm), out of which 35% may further exacerbate into critical conditions, requiring respiratory aid. Mid-panel (SARS-CoV-2 Dynamics): Owing to current RT-PCR assay quality and swab collection methods, the viral load might be undetectable up until a few days later from symptoms onset. The viral load may peak at around day 10 to then lower in healing patients, or stay high in complicated subjects at the disease "turning point" at about 2 weeks. At peak, seroconversion likely occurs. IgM and then IgG start appearing in the blood at around 3 weeks and likely plateau at 4 weeks. In critically or severely ill patients' seroconversion might be delayed and the titer is unclear with time. Lower Panel (Pharmacological Treatments): Drugs and therapies can be broadly divided into those that aim to curb viral replication (antiviral effects) advised to be given at early stages of infection to flat the viral curve, and those that aim to modulate host response (immune-modulatory effects) advised as a cytokine storm remedy for severely ill patients.

antibody (10 binding sites); then IgG class molecules appear (2 binding sites). Both IgM and IgG antibodies against SARS-CoV-2 plateau around day 28 from the infection event (that is 3 weeks from symptom onset).[26] Whether seroconversion is correlated with the lowering of the viral load or the improvement of symptoms, it is still unclear.

Depending on the host's preexisting conditions and immune response capabilities, immune response and standard care (including oxygen therapy) might not suffice to fight the infection. In these individuals, COVID-19 progresses to severity at later phases of the infection time-course. In this situation, the viral load may persist at very high levels with a subsequent massive host-cell lysis that could trigger the cytokine storm. In severe and critical patients, viral dynamics is still elusive, but shreds of evidence indicate a persisting respiratory viral load and an undeveloped and/or delayed antibody response.[20,26,27]

Viral antigen-specific immune response unpredictability, together with an apparent slower-than-anticipated antibody induction, provides a strong rationale to rely on pharmacological intervention. According to the viral load dynamics and time-course, an antiviral strategy should be deployed at early symptom onset (ideally before the peak), especially in individuals who are exposed or at a high chance of exposure. An antiviral strategy, either as prevention or treatment, at an early phase, might flatten the viral load, thus reducing the risks associated with the COVID-19 disease progression. If the antiviral window is missed, then antiviral usage would likely be less effective in the case of sustained peak levels. In this situation, the disease evolves to a point where it mounts a disproportionately strong innate immune response (viz., cytokine

storm). Therefore, immunomodulatory drugs should be used to quench the cytokine storm to avoid tissue damage and multiple organ failure. Having formed an introductory body of knowledge about the virus and disease and having positioned each class of pharmacological intervention (antiviral vs. immunomodulatory) in the appropriate timeframe, we will next discuss each pharmacological class and its most important available candidates, at present.

Pharmacological Strategies: Antivirals and Immunomodulators

Antivirals

Antiviral drugs are compounds intended to disturb the viral cycle (see Mechanisms of Infection). The intent is to reduce the magnitude of the SARS-CoV-2 load, and, perhaps, to completely suppress viral replication, allowing the development of protective immunity against the virus. Currently, there are no compounds designed as an anti-SARS-CoV-2 drug, nor is there is sufficient time to design one with proven safety and preliminary efficacy. Therefore, compounds intended for other indications, with demonstrated potential for SARS-CoV-2, are being repositioned to COVID-19. Drug repositioning can be a much more efficient entry into clinical studies. Drugs in advanced studies or that are already approved not only have general safety information available, but their manufacturing processes are well-defined. In the following, we categorize antivirals based upon their putative pharmacologic actions and checkpoints in the viral replication lifecycle.

Viral Entry Inhibitors

The first step in infecting human host cells involves binding to the cell's Angiotensin-converting enzyme-2 (ACE2) with the SARS-CoV-2's spike protein S in the trimeric conformation. As successful contact with the cell is essential to gain entry into host cells, the interfering agent blocking this step represents an intervention point to abort infection altogether. There are clinically approved drugs that inhibit ACE2 to treat hypertension called ACE-inhibitors (ACEi) and angiotensin-receptor blockers (ARB). Thus, it is thought that ACEi and ARB may compete with virus spike protein to inhibit viral entry and the subsequent infection of human lung cells. However, spike viral proteins appeared to upregulate ACE2 expression in animal lung cells and thus raised concerns about whether receptor overexpression induced by ARBs and ACEi could enhance viral entry.[28] On the other hand, controlling blood pressure can be useful against cytokine storm effects. A list of ongoing ACEi and ARBi under clinical investigation for COVID-19 is listed in Table 1. Other compounds that are thought to inhibit viral entry, such as *chloroquine* derivatives, *nelfinavir,* or monoclonal antibodies, are also discussed later.

Protease Inhibitors

The main SARS-CoV-2 protease (M^{pro}) is considered a promising drug target, as its properties are unique and distinct from human proteases. Scientists believe that inhibition of SARS-CoV-2 proteases is key to reducing viral replication and viral load.[29] The sequence and structure of this protease have been reported to be similar to that of other CoV, and therefore facilitates the search for inhibitors and compounds for this drug target through repositioning from existing compound libraries. The inhibitor of HIV protease, *lopinavir,* was found to inhibit SARS-CoV-2 protease and viral replication at a therapeutically meaningful range. *Lopinavir* affinity for CoV's M^{pro} was computationally confirmed in several docking assessments.[30,31] *Lopinavir* (in combination with *ritonavir*) was thus deployed immediately around the world for preventing SARS-CoV-2 infection and disease progression.[32]

An early study result for *lopinavir-ritonavir* on hospitalized COVID-19 (moderate-severe) patients indicates that *lopinavir-ritonavir* appeared to have no clear impact in reducing the duration of patients in ICU or need for mechanical ventilation.[33] However, in a more recent randomized study enrolling patients at early symptom onset in Hong Kong (n = 127, recruited within 7 days from symptom early onset), the effects on early treatment with *lopinavir-ritonavir* may have shown survival benefits. In this study, the investigators compare *lopinavir-ritonavir vs. lopinavir-ritonavir* plus *ribavirin* (a broad-spectrum antiviral) and IFN-beta.[34] When *lopinavir-ritonavir* was given either alone or in combination with *ribavirin* and IFN-beta, no patients died in either treatment group, while no difference in adverse effects outcomes was observed between groups.[34] The addition of *ribavirin* and IFN-beta to *lopinavir-ritonavir* did not deliver a significant impact but provided general symptoms relief and more rapid recovery time. Therefore, the role of *lopinavir-ritonavir* for COVID-19 patients with mild or moderate symptoms in the early course of viral infection could be beneficial. But ongoing clinical studies may shed light on how and when this therapy should be used to provide optimal effects with safety. In waiting for conclusive results about *lopinavir-ritonavir*, its usage is thus considered off-label, because to date *lopinavir-ritonavir* is not approved by the U.S. FDA as a treatment for COVID-19.

Other HIV protease inhibitors, including *darunavir* and *nelfinavir,* are also being examined. In a small RTC of 30 subjects, *darunavir* was reported as not promising in resolving the disease in patients admitted with moderate symptoms after 5 days of treatment vs. standard of care.[35] *Nelfinavir* exhibited very high potency in vitro against SARS-CoV-2.[36] It has been hypothesized that *nelfinavir* acts not only to inhibit protease but also to inhibit cell fusion by the spike.[37] *Nelfinavir* is awaiting clinical setup, so as of now, we can only express interest in its potential (see Section Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention for additional details about *nelfinavir* pharmacokinetics). Overall, while more potent and SARS-CoV-2 protease-specific inhibitors are being sought, repositioning HIV protease inhibitors, particularly the combination *lopinavir-ritonavir* may be the best option available for inhibiting SARS-CoV-2's protease activity. Ongoing clinical studies should provide clarity about a regimen that can achieve an optimal therapeutic response, while modeling can guide the choice (See

Table 1

List of Repositioned FDA-Approved Drugs and Traditional Chinese Medicines Under Clinical Evaluation for COVID-19 Organized by Type of Effect Elicited (Antiviral vs. Immunomodulatory).

Pharmacological Effects			
Viral Infection	Clinical Trials	Immunomodulation	Clinical Trials
	N		N
Antivirals		Corticosteroids	
CQ or HCQ[a]	201	methylprednisolone	17
lopinavir-ritonavir	40	dexamethasone	16
remdesivir[b]	24	hydrocortisone	5
ribavirin	5	Interferons[a]	
nelfinavir	n/a	IFN-beta	11
ACEi and ARB		IFN-alpha	7
Losartan	8	IFN-lambda	4
Valsartan	1	IL-6 blocker	
Broad-spectrum Neutralizing Ab		tocilizumab	30
Convalescent Plasma	136	sarilumab	9
Traditional Chinese Medicine[a]		siltuximab	2
Qingfei Paidu	4	IL-1 blockers	
Xiyanping Injection	4	Anakinra	16
Lianhua Qingwen	2	JAK inhibitors	
Ma Xing Shi Gan	2	Baricitinib	13
Tanreqing	2	Ruxolitinib	12

FDA-approved drugs in COVID-19 clinical trials globally registered in *ClinicalTrials.gov*. Drugs approval status checked in FDA-approved drugs database, https://www.accessdata.fda.gov/scripts/cder/daf/. TCM medicines accessed from http://www.chictr.org.cn/index.aspx. Websites accessed in September 2020.

ACEi, Angiotensin-Converting Enzyme inhibitors; ARB, Angiotensin Receptor Blockers; JAK, Janus Kinase; IL, Interleukin.

[a] Both antiviral and immunomodulatory action.

[b] Approved for hospitalized COVID-19 adult and pediatrics (3.5–40 kg, or <12 of age weighing >3.5 kg).

Section Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention).

RNA Polymerase Inhibitors

Inhibiting the SARS-CoV-2 RNA-dependent RNA-Polymerase (RdRP) enzyme is also a potential strategy to stop viral replication. Drugs that inhibit the HIV reverse transcriptase step have been proven to lower viral loads and enable HIV patients to live a quality life; in this way, HIV has changed from a deadly to a manageable chronic disease.[38] Among the RdRP inhibitors tested, *remdesivir* exhibits a high potency (nanomolar range) against MERS-CoV as well as SARS-CoV-2 in vitro and in animals.[39] Due to the recent containment of the Ebola outbreak, there is a large stock of *remdesivir* and a good manufacturing process. Following an initial use under emergency use authorization (EUA) for treating patients with severe COVID-19 disease, it has gained on October 22, 2020, the FDA approval for adults and pediatric patients (hospitalized weighing 3.5–40 kg, or <12 of age weighing >3.5 kg) for the treatment of COVID-19 requiring hospitalization. *Remdesivir* should only be administered intravenously (IV) in a hospital or in a healthcare setting capable of providing acute care comparable to inpatient hospital care. In a randomized controlled clinical trial study (n = 237), where patients with severe COVID-19 were treated concomitantly with *lopinavir-ritonavir*, interferons, and corticosteroids, the time to clinical improvement was evaluated. The *remdesivir* arm was not associated with a difference in time to clinical improvement (although a trend in clinical improvement in those treated earlier was observed).[40] While waiting for the final report for a larger study, another interim report indicates that the use of *remdesivir* provides a significant reduction in time to recovery for patients on ventilators compared to those in saline control by 2–3 days.[41]

Another antiviral drug *ribavirin* can serve as an RdRP inhibitor. It is indicated for chronic hepatitis C and it is used in several viral infections of the respiratory system (e.g., RSV). Although characterized by a low in vitro potency against SARS-CoV-2,[42] as previously mentioned, it is a confounding factor in a combination study that improved clinical recovery time in non-critical patients. Nonetheless, it does not seem likely that *ribavirin* can meaningfully impact COVID-19 treatment.[34]

Multi-Function Agents

Several agents that may influence multiple pharmacological and immunological pathways in viral infection and host responses are being tested. These agents include inhaling therapies with and without steroids to improve ventilation in COVID-19 patients, or non-specific supplements, such as vitamin D, C, or stem cells, for a total of 30% of the global clinical trial effort (summarized in Fig. 3). We will not include them in our discussion, as the pharmacological basis for these agents is not yet fully characterized. Thus, only selected agents are discussed below.

Chloroquine and Hydroxychloroquine. Originally discovered and used as an anti-malarial agent, *chloroquine* (CQ) and its hydroxyl derivative *hydroxychloroquine* (HCQ) have been used as immune-modulatory agents to treat autoimmune conditions such as psoriasis, arthritis, and lupus. Thus, their immunomodulatory functions have been clinically validated and prescribed world-wide by rheumatologists. The information available to date suggests that CQ and HCQ inhibitory responses in viral replication involve multiple mechanisms at the cellular and systems levels: (I) inhibiting entry by preventing the glycosylation of ACE2 cell surface receptors (entry blocker); (II) inhibiting acidification of the endosome to prevent viral RNA release; (III) augmenting antigen processing for MHC class I and II presentations to enhance immune response; (IV) enhancing the activity of regulatory T cells. Because of HCQ's putative multiple

effects against the virus and cytokine storms, plus worldwide availability at low cost, it was approved under emergency use authorization (EUA). However, preliminary results reported recently from an unblinded RCT in patients with COVID-19 (mild-critical) admitted to 176 centers in the UK did not yield positive outcomes. In this report, 418 were given HCQ and 788 were allocated to standard care. The 28-day study found that HCQ did not reduce mortality but increased the length of hospital stay and increased the risk of progressing to invasive mechanical ventilation or death.[43] Because of these negative outcomes on safety and a potential increase in hospital stay,[43] along with other similar discouraging results,[44,45] as well as safety concerns on QT-elongation at elevated doses, the FDA revoked the EUA of CQ and HCQ as a COVID-19 treatment on June 15, 2020. We are still waiting for a conclusive decision about when and who should benefit from HCQ as its low cost and high accessibility still represent an attractive option.

Virus Neutralizing Therapy

Because biologics based upon recombinant antibody production at the pharmaceutical scale may take some time to develop, scientists have been testing the century-old idea of using antibodies in serum collected from those who fully recovered. This passive immunotherapy is being tested via RTC studies. In an interim report of 81 subjects, 38 received convalescent plasma (CP) and the remainder received standard care. It showed that mortality and progression to respiratory aid rates were zeroed with CP vs. 9% and 14%, respectively, of those under the standard of care.[46] However, the definitive impact of CP on COVID-19 disease is still under evaluation. Some outstanding questions include whether the pooled plasma of COVID-19 subjects (from which CP product is made) has sufficient neutralizing antibody titer to impact outcomes of patients, or if/how the healed individual will maintain their antibodies over sufficient time during infection is still unknown. A recent study from Iceland suggests that the SARS-CoV-2 antibodies may not decline for at least 4 months.[47]
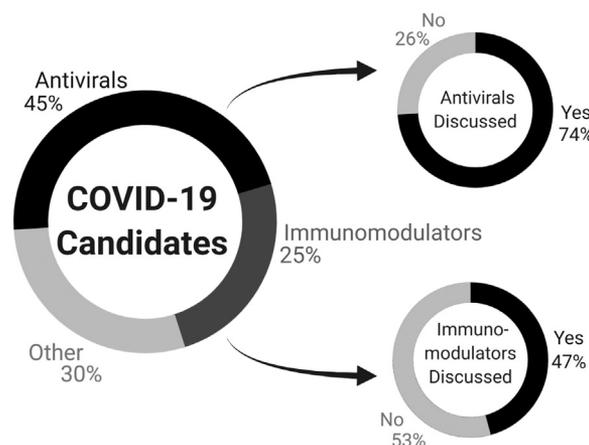
Fig. 3. COVID-19 clinical trials registered in www.clinicaltrials.gov including drugs (alone or in combination) that are discussed in this paper and divided by effects elicited. Count of all the registered clinical studies about COVID-19 (all status, www.clinicaltrials.gov, accessed Sep 2020), that are discussed or mentioned in the text, or reported in Table 1. Panel a: The proportion of repositioned antivirals is 45%, while those repositioned as immunomodulatory compounds are 30%. The remaining 30% of clinical trials contain agents pharmacologically not well-defined and therefore denoted by "other" (e.g., vitamins, oxygen therapy, stem cells, etc.). Panel b: Percentage of clinical studies about antiviral drugs that we cover in the text and report in Table 1. We discuss/mention 74% of the total antivirals clinically tested. Panel c: Percentage of clinical studies about immunomodulatory drugs that we cover in the text and report in Table 1. We discuss 47% of the total immunomodulators clinically tested.

Recently, at least two monoclonal antibody products have undergone clinical study with the intent to neutralize the virus during the early course of SARS-CoV-2 infection. One of these called *LY-CoV555* (*bamlanivimab*) received FDA approval under EUA for a single subcutaneous 700 mg dose under the outpatient setting. The *LY-CoV555* appears to be able to neutralize Italian and Washingtonian isolate of SARS-CoV2 around 0.2—4.0 μg/mL concentration.[48] Other neutralization monoclonal antibodies are at earlier stages in clinical trials but likely to be approved under EUA in a near future. The availability of effective and safe monoclonal antibodies, while more costly, could provide an additional tool to ameliorate COVID-19 disease progression.

Summary of Antivirals in Clinical Evaluation

In this paper, we divide the pharmacological strategies into two classes: antiviral and immunomodulatory. Currently, all clinical studies intended for COVID-19 are registered online on *ClinicalTrials.gov*. Based on the total clinical trials, 45% and 25% entail the usage of a compound as an antiviral or immunomodulator, respectively (Fig. 3). Of the antivirals, we mention herein 74% of the registered trials. Therefore, the discussed antivirals cover a large spectrum of leads for COVID-19. Ideally, antiviral therapy should be considered before symptoms or at mild/moderate symptom onset, up to the viral peak at day 10 (see Fig. 2). Apart from some positive signals for *remdesivir*, monotherapy has been shown to provide limited efficacy in clinical studies, especially in moderate-severe patients. From HIV experience, ideally, combinations of antivirals acting upon different viral cycle points may lead to better performances. To reduce the duration of viral shedding (correlating with transmissibility) and the risk of viral rebound (generally linked to the virus becoming drug-resistant), an optimal treatment would likely include a combination of drugs targeted to multiple viral proteins, such as a protease inhibitor, e.g., *lopinavir* (plus *ritonavir*), and a polymerase inhibitor, e.g., *remdesivir*. Furthermore, interventions like monoclonal antibodies against SARS-CoV-2 aiming to block entry or kill virions could rapidly reduce viral load and thus serve as useful additions to the antiviral armamentarium.

Pharmacological Strategies Based on Immunomodulatory Effects

As mentioned above, disease development and progression after SARS-CoV-2 infection include a later stage of tissue damage due to systemic infections beyond lung cells (Figs. 1d and 2). As a result, overstimulated host native (and mostly non-SARS-CoV-2 specific) immune results lead to cytokine storm syndrome. In these patients, impaired SARS-CoV-2 clearance, lower levels of interferons (which are generally involved in interfering with the success of virus infection to host cells), increased neutrophil extracellular traps, increased pyroptosis, and other unknown mechanisms are biomarkers reported to be associated with severe disease courses in patients experiencing a cytokine storm.[13] Once a cytokine storm occurs, anti-viral treatments alone may not be sufficient to reduce the time-to-recovery endpoint or even reverse the course allowing the patient to recover. Thus, antiviral therapy may be combined with appropriate immunomodulatory agents to counteract the massive load of cytokines in the body. We will discuss in the following section cytokine-directed therapies intended to remedy dysregulated inflammatory signaling pathways.

Corticosteroids

Corticosteroids are well-tested immunosuppressive and anti-inflammatory agents that can potentially function as anti-cytokine storm agents. Corticosteroids bind to the glucocorticoid receptor, inhibiting pro-inflammatory cascades, thus promoting anti-inflammatory signals. Thus, the promising role of *dexamethasone* in lessening the death count in intubated subjects has already been reported. In 6425 moderate-to-critical COVID-19 patients, 2104 were treated with *dexamethasone* vs. 4321 in standard care. Compared to those in the standard of care, the death rate of COVID-19 patients treated with *dexamethasone* was reduced by one-third in ventilated patients and by one-fifth in other patients receiving oxygen only; however, there was no benefit for those patients who did not require respiratory support.[49] These are very encouraging results when using this inexpensive and well-disseminated medication. Nonetheless, controversy exists about the use of other corticosteroids. Neither *hydrocortisone* nor *methylprednisolone* has demonstrated clinical improvements in critical patients.[50,51] Additionally, concerns persist regarding the risk of the early use of steroids interfering with normal immune responses.[52]

Interferon Supplementation

Interferon-beta-1a (IFN-beta), in contrast to IFN-alfa (which is more associated with antiviral effects), has been demonstrated to down-regulate inflammatory responses leading to cytoprotecting on oligodendroglia in multiple sclerosis patients.[53] By reducing vascular leakage, IFN-beta modulates cytokine/monocyte trafficking. IFN-beta may also have an antiviral effect: IFN-beta induces the interferon-stimulated genes in the nuclei that should slow down viral replication.[13] It is thought that IFN-beta could reduce the clinical impact of cytokine storms. Recently, IFN-beta was used in combination with *lopinavir-ritonavir* plus *ribavirin* to lower the recovery time for mild-moderate COVID-19 patients.[34] In a recent study, the effects of IFN-beta alone vs. standard of care were evaluated in COVID-19 patients enrolled in the hospital. The results indicate that IFN-beta significantly decreased 28-day mortality (especially if given early), as well as significantly increased the recovery rate (average recovery was 14 days from admission).[54] Interestingly, when antiviral IFN-alfa-2b plus *ribavirin* was compared to *lopinavir-ritonavir,* investigators reported no significant difference between groups in mild-moderate individuals, seemingly downgrading the role of IFN-alfa for COVID-19 [55].

Therapeutic Proteins (IL-Blockers)

Cytokines, originally discovered as cell-to-cell communicating factors found within the tissues and cells, are nearly undetectable in plasma. However, accumulated clinical data suggest that disproportionate and elevated pro-inflammatory cytokine and interleukin levels are found in plasma (viz., cytokine storm), which correlates with severe respiratory failure in COVID-19.[13] Pro-inflammatory interleukins are IL-1, 2, 6, 8, 10, 18, and 33 [13]. Among ILs, the most critical is thought to be IL-6, which is typically associated with an inflammatory state. *Tocilizumab* (H-LaRoche, CH) is an IL-6R-specific monoclonal antibody that inhibits IL-6 function and is approved for rheumatoid and juvenile rheumatoid arthritis, Crohn's, and Castleman's diseases. Numerous observational SARS-CoV-2 studies point to a general clinical improvement due to a marked decline of pro-inflammatory markers in critically ill patients by *tocilizumab* administrations.[56—58] In a recent small RTC study (n = 65, 33 in *tocilizumab* vs. 32 control), however, *tocilizumab* did not increase the cure rate but it did improve oxygenation in patients experiencing bilateral pulmonary lesions.[59] Agents intended to block another inflammatory interleukin response called *anakinra* (an IL-1 receptor-linked to IgG Fc domain), are being tested. Inhibition of JAK pathways (see Section Mechanisms of a Cytokine Storm) is also under clinical evaluation via the use of *baricitinib* and *ruxolitinib*: the former is repositioned from rheumatoid arthritis; the latter, is repositioned from high-risk myelofibrosis. Note that *baricitinib*, in combination with *remdesivir*, has just gained EUA for the treatment in hospitalized patients requiring respiratory support. It is reported in Table 1 a list of IL-blockers.

Summary of Immunomodulatory Therapeutics in Clinical Trials

In our discussion, we have included 47% of the interventional immunoregulatory agents intended (FDA-approved and repositioned) for COVID-19 (Fig. 3). Based on the time-progression from infection to disease development (Fig. 2), pharmacological or biological agents intended to modulate host (non-viral specific) immune responses may perform best at later stages of the disease, especially when a cytokine disease is diagnosed. The overproduction of IL-1 and IL-6 could lead to a range of untoward systemic effects that should be counteracted by IL-blockers to provide general relief and better oxygenation (e.g. *tocilizumab* or *baricitinib*). Currently, *dexamethasone* is the only medication with a demonstrated reduced death rate based upon RTC studies in patients in critical condition (requiring breathing support). Nonetheless, in the early stages, the emerging evidence seems to point to a positive outcome when using IFN-beta (both as antiviral and immunomodulator) with/without antivirals.

Traditional Chinese Medicine as Adjuvant Therapy

Traditional Chinese medicine (TCM) has a history of over 3000 years and an accumulated clinical practice in handling past viral epidemics. TCM involves the use of inexpensive herbs that could have both antiviral and immunomodulatory effects. Some authors believe that TCM focuses on improving blood circulation, while others believe that TCM exerts pharmacological effects. During the SARS-CoV-1 crisis in China, TCM played an important role in complementing disease management with Western approaches to clinical care. TCM use has been reported to correlate with positive clinical effects, such as better control of fever and lesser usage of steroids.[60] Although drug interactions with traditional Chinese medicines may occur through the main pathway of cytochrome P450 enzymes, it is reported that 92% of Chinese COVID-19 patients have received supplements from TCM without increasing adverse drug reactions to Western medicine regimens.[61] In Table 1 we report TCM candidates included in clinical trials as supportive medicines. For example, *Lianhua Qingwen* is a medicine composed of 13 herbs that are commonly used to treat seasonal influenza with an in vitro potential for other pathogenetic CoV.[62] Integrating Western and TCM for COVID-19 disease management may improve both hospitalized and home patient's quality of life.

Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention

The quantitative aspect of the Systems Pharmacology is also known as Quantitative Systems Pharmacology or QSP. QSP is a set of modeling and simulation tools that integrates biological pathways, cells, and organs up to simulating the whole body (e.g., PBPK). QSP and PBPK were initially used as computational methods to fill the knowledge gap and improve the translation of drugs in vivo and in humans. Currently, they are applied to de-risk drug discovery and drug development research by integrating financial elements, such as reducing animal use or optimizing dose selection for safe-and-effective therapies. To make significant progress and accelerate the global effort to find effective and safe COVID-19 treatments, QSP could assist with drug choice, timing, and dosing as well as identifying drug combinations likely to provide added benefit. QSP and PBPK models, when validated with clinical data, allow simulations of changing inputs, which would let the clinician envision an optimized design of trials and fill the knowledge gap surrounding drug repositing in emergency settings. In the following, we will discuss modeling applications leading to the repurposing of effective-and-safe drugs or drug combinations for COVID-19 treatment.

At a minimum, it is clear that SARS-CoV-2 infections in the lung cells can lead to severe respiratory failure and therefore pharmacological agents must provide sufficient levels in the lungs. However, clinical pharmacokinetic studies typically describe only plasma time-courses. In a general absence of distribution data in the lungs (e.g., HIV drugs), and in current settings where the experimental collection of drugs in the lungs will take time and it is hard to obtain, PBPK modeling, which can model the drug distribution in all the organs in the body, can provide in silico predictions of the drug concentration in the lungs. PBPK modeling will be an invaluable tool to predict likely antiviral effects of an experimental drug candidate for optimal strategies, ideal dosing, and timing for clinical evaluation, which can then be adjusted once lung tissue analysis in COVID-19 patients' data are available.

Application of PBPK Modeling in Drug Repositioning in COVID-19

The repositioning of drugs intended for other diseases (drugs either already approved for use or terminated during preclinical and clinical testing) can have two main advantages. First, manufacturing should be already in place. Second, these compounds may have significant preclinical and clinical data about safety and efficacy as well as checked dosages and frequency. This could be invaluable data to extrapolate safe regimens for treating COVID-19. The setback in drug repositioning, however, could be to elaborate a new dose or regimen that is effective in the COVID-19 target tissues. For instance, *lopinavir-ritonavir* is repositioned in COVID-19 adopting the HIV-prescribed regimen Kaletra (BID 400:100 mg), even though Kaletra may not target sufficiently the lungs against SARS-CoV-2. To investigate new targeting strategies in drug repositioning, PBPK modeling can represent a handy tool: we can compare in vitro 50% maximal SARS-CoV-2 inhibitory effect concentrations (EC_{50}), with both average range plasma concentrations (C_{max} and C_{min}) and PBPK-based predicted concentrations of the drug candidate in the lungs ($C_{max, lungs}$ and $C_{min, lungs}$). In vivo efficacy of a repositioned drug can be further assessed by an Inhibitory Quotient (IQ_{50}), i.e., the ratio between minimal projected lung concentration $C_{min, lungs}$ and EC_{50}. IQ_{50} should ideally be greater than unity when extrapolating in vitro to in vivo inhibitory effects for an antiviral.[63] For PBPK simulations, we took advantage of an online and validated platform brought by the CERTARA COVID-19 Modeling Consortium (CERTARA, NJ). They use PBPK and population PK approaches (popPBPK-PD models).[16] Because antivirals may be easier to reposition and they can be promptly deployed to flat the viral load, we discuss in the next section modeling-based repositioning of antivirals (classified upon the mode of action), thus leaving immunomodulators or monoclonal antibodies repositioning for future work.

Protease Inhibitors (Lopinavir, Ritonavir, and Nelfinavir)

The presence of *lopinavir* in fluid and cells obtained from bronchoalveolar lavage of HIV-infected patients receiving *lopinavir-ritonavir* was demonstrated,[64] but it is unknown whether current HIV-based regimens are sufficiently tackling SARS-CoV-2. The EC_{50} literature aggregated value against SARS-CoV-2 is 12 µg/mL for *lopinavir*. At the steady-state, Kaletra oral dosing reports plasma max-min concentrations in the EC_{50} range (18−5.0 µg/mL, Table 2). When simulating the lungs, max-min *lopinavir* concentrations are higher than the plasma (28−9.0 µg/mL, Table 2). The Kaletra's Inhibition Quotient ($IQ_{50} = C_{min, lung}/EC_{50}$) is 0.75, which is lower than unity, and therefore Kaletra may be a moderate reposition, as its projection of in vivo efficacy might not be very promising. In the attempt to raise the plasma and lung concentrations of *lopinavir* (and IQ), we simulate a scenario where a loading dose of *lopinavir* (800:200 mg BID) is given, followed by Kaletra regimen. As a result,

Table 2
Clinical Pharmacokinetics of Select Antiviral Drug Candidates in Clinical Trials With Proven In Vitro Effective Concentrations Against SARS-CoV-2.

Drug	Route	Regimen	EC_{50}	Plasma[a]			Lung[b]		
				C_{max}	C_{min}	$T_{1/2z}$	$C_{max, lung}$	$C_{min, lung}$	IQ_{50}
Lopinavir[c]	oral	*400 BID*	12	18	5.0	3.0	28	9.0	**0.75**
Lopinavir[c]	oral	*800*[d] *& 400 BID*	12	23	6.0	6.0	32	11	**0.92**
Nelfinavir	oral	*750 TID*	0.63	3.0	2.5	4.0	0.11	0.07	0.11
CQ	oral	*1250*[d] *& 250 QD*	2.2	1.1	0.08	50	7.0	5.0	2.3[e]
HCQ	oral	*400*[d] *& 200 QD*	0.33	0.47	0.07	500	8.0	6.0	19[e]
Remdesivir	IV	*200*[d] *& 100 QD*	0.52	4.3	0	1.0	7.0[f]	2.0[f]	**3.9**

EC_{50}: Candidate half-maximal drug concentration to inhibit SARS-CoV-2 from collated in vitro literature. C_{max}: plasma drug maximum/peak drug concentration, µg/ml; C_{min} plasma drug minimum/trough drug concentration, µg/ml; $T_{1,2}$: terminal half-life, hr; $C_{max,lung}$: lungs drug maximum/peak drug concentration, µg/ml; $C_{min,lung}$ lungs drug minimum/trough drug concentration, µg/ml. IQ_{50} as the Inhibitory Quotient as $C_{min,lung}$ / EC_{50}, with $C_{min,lung}$ projected by PBPK modeling. C_{max}s, C_{min}s and $T_{1,2}$ parameters from averaging concentration-times for 5 days of treatment. Doses in mg. BID, twice a day; QD, once a day; TID, three times a day.

[a] Data collected from clinical pharmacokinetic studies in healthy adults.
[b] Lung concentrations simulated by PBPK models in a virtual healthy population of 100 subjects (CV<50%). PBPK models from.[16]
[c] *Lopinavir* in combination with *ritonavir* as 4:1 molar ratio.
[d] Loading dose.
[e] It may not translate into clinical effectiveness. Refer to *Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention — Chloroquine and Hydroxychloroquine*, for more details.
[f] Based on *remdesivir's* total nucleoside metabolites *GS-441524s* in SARS-CoV-2-diseased rhesus monkey lung lobules homogenate collections[80], as a surrogate measure of prodrug *remdesivir* activity. Refer to *Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention — RdRP Inhibitors (RdRP)*, for more details.

the drug increases in plasma as 23−6.0 µg/mL with an improved predicted lung concentration of 32−11 µg/mL, which yields a better IQ_{50} = 0.92, and therefore this may be a more suitable regimen (Table 2). Nonetheless, it should be noted that *lopinavir* repositioning and modeling is hindered by several factors: (I) *lopinavir-ritonavir* generates variable pharmacokinetics probably due to circadian plasma clearance patterns and enzyme inductions.[65,66] As the label and other clinical studies suggest, *lopinavir* is high in the first week of treatment and then it drops to a lower steady-state level[67,68]; (II) *Lopinavir* is a highly protein-bound compound. To address the protein binding effects in an in vitro-to-in vivo extrapolation, *Thakur* et al. simulated lung unbound concentrations of *lopinavir* and compared them to unbound-corrected EC values, which were computed from HIV experiments. Their results suggest that 1400:350 mg QD or 1000:250 mg BID regimens could get near to efficacy levels in the lungs, denoted by EC_{50} but not by EC_{90}, in either Caucasian or Chinese virtual populations.[69] However, we recognize that these supratherapeutic doses of *lopinavir-ritonavir* proposed by *Thakur* et al. may bring adverse events, including cardiotoxicity and gastrointestinal effects (C_{max} > 25 µg/mL[70]).

An important but often missed piece of information about the combination *lopinavir-ritonavir* is that *ritonavir* is thought to function mainly as an oral pharmacokinetic booster for *lopinavir*. Despite this, *ritonavir* might have moderate viral replication inhibition as well. *Ritonavir* was found to potentially achieve HIV EC_{50} levels in peripheral blood mononuclear cells (PBMC) at steady-state when given in combination with *lopinavir*, but not alone.[65] In a benchmark MERS study by *Sheahan* et al., the combination *lopinavir-ritonavir* lowered the *lopinavir* EC_{50} by 37% (improved efficacy), than *lopinavir* alone.[39] Interestingly, in the same experiment *lopinavir-ritonavir* plus IFN-beta achieved a much-improved efficacy over *lopinavir-ritonavir* alone.[39] Therefore, *ritonavir* may have a double role in the combination, both as a pharmacokinetic booster and as a low-to-moderate anti-SARS-CoV-2 inhibitor.

Nelfinavir is another inhibitor of HIV protease with an additional putative role as a blocker of the viral *S*-fusion. It exhibits remarkably high in vitro potency against SARS-CoV-2 with EC_{50} = 0.63 µg/mL and EC_{90} = 1.1 µg/mL; these values are about 10-fold lower than that of *lopinavir*.[36] The current recommended dose for HIV therapy is 750 mg three times a day (TID). According to the label, *nelfinavir* achieves max-min concentrations in the range of those SARS-CoV-2's ECs levels in the plasma (3.0−2.5 µg/mL, Table 2). Although

nelfinavir was detectable in bronchoalveolar lavage fluid in HIV patients treated for 4 weeks, and the concentration of *nelfinavir* in the lung epithelial lining fluid was noticed to be similar to that of plasma,[71] PBPK modeling predicts a very low lung distribution of *nelfinavir*. Likely, physicochemical properties of *nelfinavir* in tissue composition equations, employed in the PBPK model to compute the in silico lung-to-plasma partition coefficient, generate insufficient lung concentrations for this protease inhibitor, (max-min lungs, 0.1-0.07 µg/mL, and IQ_{50} = 0.11, Table 2). Nonetheless, before concluding about an in vivo inefficient target pharmacokinetics of *nelfinavir*, we should wait for clinical results to corroborate the model predictions. In fact, in vivo hydroxylated *nelfinavir* M8 metabolite, which is not present in vitro, has activity against HIV and it may reach sufficiently high concentrations to add to the therapeutic effect. We should also be aware that the degree of accuracy in extrapolating EC_{50} or IQ_{50} from in vitro experiments to in vivo for protease inhibitors will be improved when protein-adjusted IQ_{95}s become available.[63]

In conclusion, it is anticipated that *lopinavir* exposure in the lungs might approach in vitro 50% max effects on viral replication when given at an approved dose and frequency (with loading doses). In particular, the regimen made of 800:200 BID as a loading dose, followed by the standard 400:100 mg BID thereafter, may improve target IQ_{50} and therefore could represent an optimal repositioning regimen to target SARS-CoV-2 protease activity in the early stages of infection (before the viral peak).

Viral Entry Modulators (Chloroquine and Hydroxychloroquine)

Anti-malarial *chloroquine* (*CQ*) is typically given in several COVID-19 trials as one loading dose of 1250 mg followed by 250 mg QD. As shown in Table 2, *CQ*'s EC_{50} = 2.3 µg/mL is not achievable in plasma (1.1−0.08 µg/mL). According to modeling prediction, concentrations in the lungs are in the range of the EC_{50} generating a good IQ value (7-5 µg/mL, IQ_{50} = 2.3). Instead of raising the *CQ* dose, which could lead to prolonged QT intervals (thus exposing higher risks to patients with existing heart morbidity), the hydroxy derivative *hydroxychloroquine* (*HCQ*) has shown better pharmacokinetics as well as an improved EC_{50} = 0.33 µg/mL.[72] *HCQ* is tested as a 400 mg loading dose, followed by a 200 mg QD. *HCQ* shows lower plasma levels than *CQ* (0.47−0.07 µg/mL), and although similar lung pharmacokinetics, much improved IQ_{50} results (8-6 µg/mL, IQ_{50} = 19, Table 2).

As anticipated, *HCQ* may have advantageous pharmacokinetics and improved predicted in vivo efficacy compared to *CQ*. Despite apparently high IQ_{50}s, clinical results for *CQ/HCQ* seem to indicate a lack of clinical efficacy for either COVID-19 treatment or pre-exposure prophylaxis. It is unclear the reason for this, and interestingly, despite a high in vitro potency against the Ebola virus, *CQ* also failed to translate into its in vivo effectiveness in protecting animals from Ebola.[63,73] This could be that the reported EC_{50} for *HCQ* might be highly dependent on the multiplicity of infection (MOI, the ratio between the number of viruses and the number of host cells), and when MOI is high enough, $EC_{50} = 5$ μg/mL ($EC_{90} > 15$ μg/mL),[74] which would make an *HCQ's* IQ_{90} to decrease much lower than 1, thus suggesting poor in vivo efficacy. Also, *Fan* et al. suggested that *HCQ* distribution in the whole blood hinders the real extracellular lung concentration, which is supposedly much lower than other authors believe.[75] Despite further investigation should shed light on this, if *CQ/HCQ* are still designed for COVID-19, perhaps in combination with other therapeutics, dose-ranging effects should be carefully considered due to prolonged residence in sensitive off-target tissues. Both compounds are characterized by very large volumes of distribution (500 L/kg) and very long half-lives as $T_{1/2} = 50, 500$ days in healthy volunteers for *CQ* and *HCQ,* respectively,[76] thus providing the rationale of important tissue distribution, including the heart (*CQ's* heart-to-plasma ratio in rodents is 17[77]).

RdRP Inhibitors (Remdesivir)

Remdesivir shows high potency against SARS-CoV-2 in vitro, with EC_{50} documented as 0.52 μg/mL and $EC_{90} = 1.1$ μg/mL.[3,78] *Remdesivir*, lyophilized or in solution form, is approved as an injectable dosage formulation for infusion for COVID-19, i.e., 200 mg as a loading dose and 100 mg QD thereafter. As shown in Table 2, *remdesivir* could rapidly achieve a 4.3 μg/mL plasma peak but it disappears from plasma very quickly with a half-life of about 1.0 h.[3] *Remdesivir* is a diastereomeric monophosphoramidate prodrug that undergoes metabolic activation to form the active triphosphate, *GS-443902,* intracellularly. In vivo bioactivation should occur as follow: *remdesivir* is extensively metabolized by hydrolases forming an intermediate metabolite, *GS-704277* (detectable in plasma), which is then subject to cleavage of the phosphoramidate bond resulting in the formation of the nucleoside analog monophosphate *GS-441524-MP* (phosphoramidase step). *GS-441524-MP* undergoes either almost unidirectional dephosphorylation to *GS-441524* (detectable in plasma), or further intracellular phosphorylation to the pharmacologically active nucleoside triphosphate, *GS-443902,* or *Nuc-TP*. *Nuc-TP* selectively inhibits viral RNA polymerases but not host RNA or DNA polymerases.[79] *Nuc-TP* is negatively charged, hence trapped in cells with an intracellular half-life of 36–49 h measured in PBMC.[79] In PBMC, *Nuc-TP's* minimum concentration at 24 from injection was 2.9 μg/mL which was about 370-fold over a SARS-CoV-2 clinical isolate $EC_{50} = 0.0079$ μg/mL for *Nuc-TP*.[79] Although a very high concentration of the active metabolite is expected intracellularly in the blood, it is not known if *Nuc-TP* in PBMC is correlated with a clinical efficacy against COVID-19, nor if it translates into a high intracellular concentration in the lung cells. A PBPK model for *remdesivir* is included in the EUA package to infer pediatric dosages,[3] but it is not available to predict lung concentrations. For this assessment, scientists at the National Institute of Allergy and Infectious Diseases measured in NHP detectable concentrations of metabolites of nucleosides GS-441524 (total) in lung tissue collected from lung lobes, 24 h after the last *remdesivir* treatment (max-min, 7-2 μg/mL, $IQ_{50} = 3.9$, Table 2).[80] Among *GS-441524s*, lung homogenate samples spiked with the triphosphate form, *Nuc-TP*.[80] Hence, *GS-441524* levels are taken herein as a surrogate for tissue loading and suggest

that the current dosing strategy could deliver drug metabolites to the sites of SARS-CoV-2 replication in infected animals. Significantly, they observed that NHP treated within one day from infection barely displayed any COVID-19-related symptoms.[80]

Summarizing, with the limited preclinical and clinical data available, *remdesivir* active metabolites may achieve good intracellular concentrations in vivo in the lungs as demonstrated by a good IQ_{50} viral inhibition level. Additionally, clinical and NHP evidence suggests an early commencement of the treatment (ideally before the viral peak) to obtain maximal antiviral efficacy. Note that while not generating particular adverse effects in healthy patients,[79] subjects with renal failure (eGFR < 30 mL/min), or on dialysis, or continuous Veno-Venous Hemofiltration, must not receive *remdesivir*, so other treatments should be recommended.[3]

QSP Modeling to Estimate the Therapeutic Benefits of Antiviral Therapy

The efficacy (or disease-modifying outcome or effect) of an antiviral pharmacological intervention depends on two main aspects: (I) the point of intervention in the viral lifecycle (see Fig. 1); and (II) the timing of intervention due to an evolving viral load (see Fig. 2). Efforts in the past to bring together comprehensive models coupling pharmacodynamics and viral dynamics have greatly advanced HIV virological and pharmacological understanding (see Ref[81] for an example). A similar approach has been reported for SARS-CoV-2 yielding interesting results. *Dodds* et al. used viral dynamic modeling entertaining four time-evolving entities: (I) uninfected susceptible lung epithelial cells; (II) latently infected cells; (III) productively infected cells; and (IV) the viral load in the respiratory tract.[29] These entities link with one another according to relationships occurring among players to obtain metrics/endpoints of interest: AUC (area under concentration-time curve) of the viral load in the respiratory tract (disease status); duration of viral shedding (transmissibility); and epithelial cells infected (tissue damage). The modeling outcome suggests that drugs acting upon killing virions (e.g., neutralizing antibodies) would limit tissue damage; Drugs acting on killing infected cells (e.g., protease inhibitors or IFN-beta) should be key in lowering viral shedding. RdRP effects (e.g., *remdesivir*) are also embodied in the model by a parameter representing the release rate of new virions. Drugs inhibiting the RdRP step resulted in a moderate reduction of all aforementioned endpoints. Altogether, the study concluded that: (I) multiple drug interventions should be attempted with drugs acting upon different viral lifecycle steps; (II) the time-window opportunity for an antiviral intervention should be as early as possible. These recommendations have been independently substantiated by other groups using similar QSP approaches.[82,83] QSP modeling of the dysregulated cellular pathways causing the cytokine storm should also be conceived to fill the knowledge gap and prompt further research for positioning immunomodulatory drugs in terms of dose, timing, and frequency. By incorporating cellular-scale viral dynamics and innate and adaptive immune responses, *Dogra* et al. ranked which parameters were the most controlling of the pathogenesis. Although their modeling still requires validation, in their concept they concluded about the dominant role of innate immunity due to IFNs and macrophages as well as the importance of timing when initiating therapy.[84] Overall, the conclusions from all these QSP and pharmacodynamic studies are consistent with our thinking as we have discussed in the manuscript.

Future Direction

While lockdowns, quarantines, and general social distancing were recommended or mandated to control the SARS-CoV-2

pandemic and death count, they require compliance and discipline balancing between individual liberty and the sake of the public good. Ultimately, a vaccine capable of preventing infection or disease progression will be needed to fully return to normality in public gatherings and economic activities. Early sharing of the virus's genetic sequence along with transcriptomic mapping has enabled the accelerated design and testing of COVID-19 vaccines. Several recombinant viruses that express SARS-CoV-2 proteins or RNA formulations directed to express proteins are used in the US and Europe as vaccine candidates. These vaccines do not contain viral proteins and RNA derived from the infectious virus, which is inactivated in the killed vaccine; thus, these vaccine candidates do not carry the risk of breakthrough vaccine-related SARS-CoV-2 infection. Due to the unprecedented research effort, and government investments in stockpiling promising candidates with large pharma partnerships, there are at least 6 vaccines leads, including mRNA-based, recombinant, and virus-like particles that are at the advanced Phase 2/3 testing stage.[85] Any of the vaccine candidates proven effective-and-safe in preventing infection or disease progression in at least 50% of individuals who are in high-risk groups, will be rapidly deployed. Vaccines will overcome current social and economic restrictions and they will curtail COVID-19 spread. But because vaccine dissemination, long-term efficacy, better manufacturing, and vaccination campaigns are still hurdles, a highly active therapy is still critical. We discuss next, the potential use of Systems Pharmacology when repositioning multiple drugs in terms of drug-drug interaction safety. It is followed by the conceptualization about reformulating antivirals into long-acting therapies as optimized COVID-19 treatment solutions. It is also touched the potential role of AI in the pandemic.

Drug Combination Therapy with Drug-Drug Interaction Modeling Support

As mentioned above, antiviral therapy may require the timely deployment of an antiviral combination. Polytherapy should in principle maximize viral suppression acting upon different checkpoints of the viral lifecycle. However, concomitant administrations of COVID-19 repositioned drugs could potentially lead to drug-drug interactions (DDI), with several, sometimes unpredictable, pharmacokinetic effects. For instance, the coadministration of *remdesivir* and *chloroquine phosphate* or *hydroxychloroquine sulfate* is not recommended based on in vitro data demonstrating *CQ* and *HCQ* perpetrating effects on the intracellular metabolic activation and antiviral activity of *remdesivir*.[3] Another example is *ribavirin* interacting with *lopinavir-ritonavir,* as the alleged cause of increased drug adverse reactions reported in a recent clinical study.[55] Additionally, it is hypothesized that cytokine storms might alter CYP functions leading to unpredictable DDI metabolic reactions.[86]

In the case of HIV, potent drug combinations are devised to target multiple HIV proteins. In principle, it could the thought that a similar antiviral strategy is more effective to lower the SARS-CoV-2 load sooner. In the case of the initial design and evaluation of drug-combination candidates, the first step is to check (for each drug in combination) if any existing DDI has been discovered from labels or clinical studies, which can be a lengthy scrutinization. Consultable online tools are available such as www.covid19-druginteractions. org[87] and the University of Washington's DDI Database www. druginteractionsolutions.org.[88] Interestingly, when interrogating these resources, there is no apparent DDI sign for the combination *lopinavir-ritonavir* plus *remdesivir.*

In many cases, these DDI queries for the specific drug-combination in question can assist in quantifying the pharmacokinetic impact of DDI. Alternatively, more in-depth QSP approaches, especially PBPK, can be deployed to simulate the full

pharmacokinetic profile of an expected change due to DDI. For example, PBPK can be used to identify the potential mechanisms underlying DDI: CYP, UGT, or transporter-mediated inhibition or induction are common instances of DDI. DDI for each drug-combination candidate may be reversible or irreversible, time-dependent, or time-independent. Each of these categories has a subset of mechanisms. For example, enzyme inhibition can be competitive or uncompetitive. Knowing or predicting the type of inhibition and the pharmacodynamic effects in vivo will provide scientists with an invaluable safety check ahead of time. For these reasons, PBPK is the preferred dynamic framework to flag any risk associated with practicing a drug-combination therapy regimen with apparently safe drugs, especially if drug repositioning mandates dose adjusting.

COVID-19 Treatment and Long-Acting Strategy

The term long-acting (LA) has been employed in drug delivery to cover applications in oral and parenteral administrations where a drug was reformulated to extend the duration of pharmacokinetic exposure. Generally, LA medicines involve formulating drugs in polymeric or liposome nanocarriers to provide sustained drug-release at the site of injection to provide long-acting plasma drug levels. The goal is to sustain target therapeutic concentration for periods ranging from weeks to months following a single administration. For instance, the two antiretrovirals *cabotegravir* and *rilpivirine* have been demonstrated non-inferior to the daily intake control for monthly pre-exposure and post-exposure prophylaxis (PEP) as a combination.[89] These HIV drugs are formulated independently using polymeric stabilizers in nanoparticulate formulations to provide sustained drug release from an intramuscular injection depot. Overall, long-acting injectable formulations of HIV medications hold the promise to maintain viral load suppression while reducing to a minimum the administrations.

In transforming small molecules intended for multiple daily intakes into a single-dose lasting 2−6 weeks, our laboratory has been developing drug-combination particles containing multiple (3 or 4) antiretrovirals for LA HIV therapy (DcNP platform technology). Using lipid excipients acting as a "glue", DcNP is shown to extend plasma drug concentration of the two to four drugs in formulation for 2 weeks or more in non-human primates (NHP) after a single injection. In addition, drugs given in DcNP can enhance mononuclear cellular drug uptake in the lymph nodes and the blood. Drugs delivered by DcNP are shown to be active, as the intracellular HIV drugs of nucleoside analogs, such as *tenofovir* and *lamivudine*, are able to convert into active metabolites (e.g., *tenofovir diphosphate*) with similar efficiency to that of controls with parent drugs without DcNP formulation. Antiretrovirals in DcNP have demonstrated good stability with particles thus protecting them from the native and rapid elimination, while reducing toxic accumulations, such as in the heart or kidney.[90] In vivo, DcNP transformed short-acting drugs that inhibit the HIV protease (*lopinavir, ritonavir, atazanavir*) and the reverse transcriptase (*tenofovir, lamivudine*), from half-lives of a few hours into weeks in both mononuclear cells and plasma.[90−93] Hence, DcNP is a subcutaneous delivery platform accommodating combinations of drugs with disparate physiochemical properties (e.g., water-solubility) suitable for transforming multiple daily intakes into a single-dose single-particle injection. This is why DcNP technology may hold potential for SARS-CoV-2 long-acting therapy, a concept that is not found in the literature at present. The proposal could exist for LA *lopinavir-ritonavir*, LA *remdesivir*, or the combination thereof.

To this end, we have herein studied whether DcNP technology could be utilized to transform COVID-19 drugs from short-acting into long-acting treatments. We use an exploratory QSP model

simulation in our first attempt to envision the LA transformation. The 7-day target is chosen as LA duration because *lopinavir* and *remdesivir,* are given for a minimum of 7 or 5-days treatments in COVID-19 patients, respectively. For this QSP model simulation, we employed a validated mechanism-based pharmacokinetic (MBPK) model, which has been structured with data from NHP studies (IV and SC dosing).[90] After empirically accounting for the metabolic differences between NHP and humans, we scaled the model to simulate the plasma concentration of LA *lopinavir*. According to the simulation, a single SC dose containing 800 mg LA *lopinavir* provided plasma drug levels greater or equal than the EC$_{50}$ target (Fig. 4a). If successfully developed as a LA *lopinavir* product, it could replace at least 7 days of Kaletra regimen (2–4 pills a day), while maintaining drug concentrations potentially effective against SARS-CoV-2 (lung concentrations are assumed higher than plasma based on Section Systems and Clinical Pharmacology in COVID-19 Treatment and Prevention and not pursued here). Similarly, we simulated a *remdesivir* LA transformation too. First, we identified the free part of the MBPK model with the *remdesivir* clinical pharmacokinetic data.[79] In this case, we assumed that the DcNP, which has been shown to extend the pharmacokinetics of another antiviral nucleoside analogs, *tenofovir* and *lamivudine*,[93] can enable LA conversion of the short-acting *remdesivir*. With this assumption, we have simulated the time-course of DcNP-*remdesivir* and presented it in Fig. 4b. As depicted in Fig. 4b, one single 7 mg LA *remdesivir* dose could provide plasma drug concentrations above EC$_{50}$ levels for the entire duration of a 5-day treatment. This is a further example of how QSP-based outcomes can enable the potential development of new or improved pharmaceuticals. Once successfully developed, an LA dosage form of anti-SARS-CoV-2 therapeutic could potentially provide sustained viral suppression and overcome current limitations on hospitals and care facilities in the COVID-19 pandemic. We believe that such long-acting therapeutic strategies would reduce the need for hospital stays, thus sparing resources and drugs while potentially lowering transmission rates.

Potential Role of Artificial Intelligence

Artificial intelligence (AI) is a set of automated computational methods to support data gathering, analysis, and subsequent decision making. These methods work well when Bigdata is available. In the context of research, in less than 8 months since the SARS-CoV-2 outbreak, a remarkably large number of reports (~2000/week) has formed big datasets.[17] AI may be used in drug discovery to screen leads for high affinity with SARS-CoV-2 targets. For example, the molecular design of a *de novo* molecule to potently bind with SARS-CoV-2 protease can be worked out via molecular docking and machine learning.[94] However, the AI-assisted compound will still necessitate long in vivo translation time. The AI role in later drug development, however, is yet to be determined.

Although AI for COVID-19 may be better used for data analysis (as performed in this paper to gather quantitative information), it could also find an appealing application as a diagnostic and prognostic tool. By training AI algorithms with validated lung CT or X-ray image data from COVID-19 positive patients, a computational tool can be designed to assist pathologists in forecasting the likelihood of COVID-19 paths towards severity.[95,96] Another exciting approach uses machine learning to predict COVID-19 mortality in positive patients. By selecting the 3 most-discriminant blood biomarkers in hospitalized COVID-19 patients, namely, lactic dehydrogenase (an indicator of cell death), lymphocytes (inflammatory response), and high-sensitivity *C*-reactive proteins (an indicator of inflammation), the AI computer-based method predicts the mortality of individual patients more than 10 days in advance with more than 90% accuracy.[97] Having such an informative set of tools, albeit somewhat non-specific to viral infection, could reveal unique time-dependent profiles of biomarker kinetics and dynamic profiles to assist physicians in quickly developing the most appropriate (personalized) pharmacological strategy.
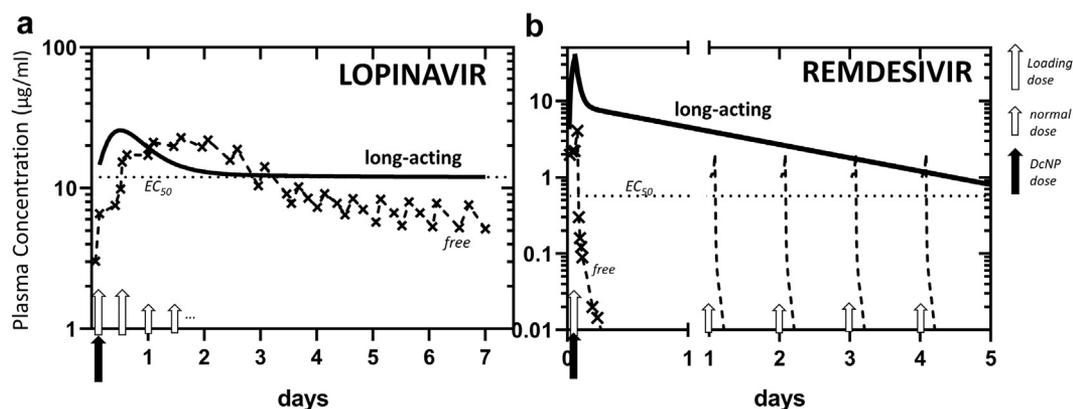

Fig. 4. Graphical presentation of the impact of transforming *lopinavir* and *remdesivir* into long-acting injectables based on a platform technology called Drug-combination Nanoparticle (DcNP) developed for HIV drug combination therapy; DcNP projections are derived from QSP-based simulation using a validated MBPK model and extrapolations to humans. Current experimental COVID-19 treatments for lopinavir and remdesivir call for a min of 7 and 5 days, respectively. Concentration-times in the plasma of lopinavir and remdesivir are depicted either as standard formulations (dashed lines with clinical data marked as "x") or conceptualized in Drug-combination Nanoparticles (DcNP, bold solid lines), vs. reported in vitro concentration of a drug that gives a half-maximal response (EC$_{50}$, dot lines). Panel a: comparison of a week of therapy with lopinavir-ritonavir, either as standard oral dosage (free, dashes with x marks) or formulated into DcNP (solid lines). In the oral free therapy, COVID-19 individuals are dosed with two loading doses (Table 2, 800-200mg, longer arrows), followed by standard Kaletra treatment (400:100 mg, smaller arrows) twice a day for the remaining 6 days (displaying non-linear pharmacokinetics). When DcNP-lopinavir (long-acting) plasma concentration-time is simulated via an HIV MBPK model a single-dose subcutaneous administration (800:200mg lopinavir-ritonavir, full arrow) could potentially provide an extended above-or-equal EC$_{50}$ plasma level for the entire week. Panel b: comparison of 5-days therapy with remdesivir, either as standard IV infusion (free, dashes with x marks in the first dose, dashes only in the following administrations) or formulated into DcNP (solid lines). In the free IV therapy, COVID-19 individuals are dosed with one loading dose (Table 2, 400 mg, longer arrow), followed by standard IV treatment (200mg, smaller arrows) once a day for the remaining 4 days. When DcNP-remdesivir (long-acting) plasma concentration-time is simulated via an HIV MBPK model a single-dose intravenous administration (7 mg remdesivir, full arrow) could potentially provide an extended above-EC$_{50}$ plasma level for the entire 5-days period.

Conclusions

Since its discovery in late 2019 as a novel pathogenetic coronavirus, the SARS-CoV-2 virus is far from being defeated. Owing to its high transmission rate, poorly recorded herd immunity, and an unclear possibility of re-infection, SARS-CoV-2 is projected to be eradicated in years. Therefore, effective pharmacological therapy is and will be the frontline to reduce infection rates and disease progression, especially for those who are exposed but not immune, regardless of the timing and availability of a vaccine. At early stages, effective antivirals are ideal for use in pre/post-exposure or at clinically mild symptom presentation. If antiviral therapy is insufficient or the disease has already progressed toward severity, immunomodulators should ameliorate the patient's over-reactive immune status. Thus, there are hundreds of ongoing clinical trials focusing on these two categories of drugs. To accelerate the timeline to access effective drugs, clinical trials are based on the idea of repositioning FDA-approved drugs from other indications. They should demonstrate inhibition of SARS-CoV-2 at plasma and lung concentrations that are achievable with currently recommended dosing. This PK-driven thinking is substantiated by the viral dynamic time-course we have collected: antivirals should flat the viral load curve before it peaks, while immunomodulators should reduce tissue damage caused by the cytokine storm. Therefore, antiviral therapy with prompt testing (assisted by AI-tools) is the best-positioned strategy to kill the virus before harming patients and avoiding subsequent ICUs clogging. Although *remdesivir* is the only recommended drug used in hospitalized patients, we have reasoned here that a *lopinavir-ritonavir* plus *remdesivir* combination might potentially offer the best antiviral option available at the moment (with the possibility of IFN-b supplementation, e.g., ACTT-3 trial, or TCM as adjuvant therapy). We supported this idea via modeling of plasma and lung pharmacokinetics coupled with the literature available regarding QSP and pharmacodynamic modeling, which integrate the viral load time-course. Furthermore, a novel pharmaceutical concept is envisioned here. For the first time in the COVID-19 literature, we have proposed the idea of transforming current antiviral regimens into single-dose long-acting injectables. By reformulating antivirals into Drug-combination Nanoparticles, the new treatment has the potential to replace 5—7 days' worth of pills or infusions. A rendering of LA *remdesivir* has the potential as a "flu-shot-like" injectable product to cover or treat patients for a 5-days or one-week treatment, possibly without the need for hospitalization.

Given the rapid, massive, and evolving information reported over the past months, it is important to implement systems thinking to develop and optimize therapeutic strategies that could overcome the social distancing necessary to flatten the curve of viral transmission and halt death in our communities. We have presented in this text the accumulated knowledge about virology, host-virus interaction time course, and disease progression in the context of Systems Pharmacology and modeling analysis. This manuscript advises the use of QSP and PBPK modeling to guide the choice and use of repositioned therapeutic agents, either alone or in combination. Overall, Systems Pharmacology thinking using state-of-the-art computational and long-acting methods can significantly contribute to the treatment of COVID-19 and it can represent an invaluable asset to combat future pandemics.

Acknowledgments

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