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Untapping host-targeting cross-protective efficacy of anticoagulants against SARS-CoV-2

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ABSTRACT

Responding quickly to emerging respiratory viruses, such as SARS-CoV-2 the causative agent of coronavirus disease 2019 (COVID-19) pandemic, is essential to stop uncontrolled spread of these pathogens and mitigate their socio-economic impact globally. This can be achieved through drug repurposing, which tackles inherent time- and resource-consuming processes associated with conventional drug discovery and development. In this review, we examine key preclinical and clinical therapeutic and prophylactic approaches that have been applied for treatment of SARS-CoV-2 infection. We break these strategies down into virus- versus host-targeting and discuss their reported efficacy, advantages, and disadvantages. Importantly, we highlight emerging evidence on application of host serine protease-inhibiting anticoagulants, such as nafamostat mesylate, as a potentially powerful therapy to inhibit virus activation and offer cross-protection against multiple strains of coronavirus, lower inflammatory response independent of its antiviral effect, and modulate clotting problems seen in COVID-19 pneumonia.

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1. Introduction

The continued emergence of respiratory viruses with pandemic potential highlights a dire need for evolved targeted antiviral therapies. Over the past 20 years zoonotic transmission has led to the appearance of highly pathogenic strains of coronaviruses (CoVs) in human populations, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and most recently severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) – the etiological agent of coronavirus disease 2019 (COVID-19). In a little over a year, SARS-CoV-2 alone has impacted tens of

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CoV, coronaviruses; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; RdRp, RNA dependent RNA polymerase; HIV, human immunodeficiency virus; ACE2, angiotensin-converting enzyme 2; Ang II, angiotensin II; M^{pro}, virally encoded main protease of SARS-CoV-2; TTSP, type II transmembrane serine protease; VSV, vesicular stomatitis virus; COPD, chronic obstructive pulmonary disease; Nrf2, nuclear factor erythroid 2 p45-related factor 2; IL6, interleukin 6; IL1B, interleukin 1B; MSC, mesenchymal stem cell; AT1, angiotensin II type 1 receptor; PD-L1, programmed death-ligand 1; FasL, Fas ligand.

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millions of people worldwide with a fatality rate of ~1–2% manifesting diversely ranging from asymptomatic infection to long-lasting respiratory impairment and organ failure (Chen, Liu, & Guo, 2020; Fernandez-de-Las-Penas, Palacios-Cena, Gomez-Mayordomo, Cuadrado, & Florencio, 2021; Israelow et al., 2020).

Unparalleled research efforts have led to development and successful implementation of multiple SARS-CoV-2 vaccines globally. While these vaccines have proven highly efficacious, the rapid emergence of novel SARS-CoV-2 variants, which are more contagious or deadly than the original strain, pose serious concerns over continued efficacy of the vaccines (Bergwerk et al., 2021; Moore & Offit, 2021). Similarly, appearance of breakthrough infections in fully vaccinated people is a growing concern (Bergwerk et al., 2021; Scobie et al., 2021). Moreover, we are facing a substantial reticence from the public on receiving vaccines with many citing concerns over their safety and efficacy leading to vaccine hesitancy (Chou & Budenz, 2020; Yigit, Ozkaya-Parlakay, & Senel, 2021). Notably, despite early indications of success, vaccination strategies utilizing innovative technologies (mRNA-based vaccines developed by Modena and Pfizer) are still relatively new and have limited safety data in humans (Dong et al., 2020). Prior studies using MERS and SARS vaccines highlighted risk of lung injury due to aberrant antibody production and development of T_h2 responses (Dong et al., 2020; Liu et al., 2019; Lurie, Saville, Hatchett, & Halton, 2020). Meanwhile, relatively limited post-marketing surveillance data on SARS-CoV-2 vaccines has been accumulated, which in part is due to the short duration since they have been implemented. However, thorough and extensive surveillance studies are required to capture a true understanding of the safety and efficacy of the approved vaccines (Dhanda, Osborne, Lynn, & Shakir, 2020). Importantly, the economic burden of mass vaccination in highly populous nations, intellectual property rights and (national and global) logistical challenges (e.g., vaccines production and distribution) further complicate utilization of vaccination strategies (Ali, Asaria, & Stranges, 2020; Lee & Chen, 2021; Rahi & Sharma, 2020; Teerawattananon & Dabak, 2020). Thus, while vaccinations remain a priority for control of SARS-CoV-2 spread, it would be negligent to rely on vaccination strategies alone, underscoring a pressing need to rapidly identify new pharmacologic agents for COVID-19. However, traditional drug development approaches can last decades and cost billions of dollars, making identification of new antivirals against current and emerging mutated strains of SARS-CoV-2 almost impractical (DiMasi, Grabowski, & Hansen, 2016; Hughes, Rees, Kalindjian, & Philpott, 2011). But repurposing clinically approved therapies for treatment of COVID-19 (Fig. 1) may provide the urgent solution needed to this debilitating pandemic. Such strategy offers at least two major benefits: (1) accumulated clinical data on safety and tolerability of therapeutics of interest (which based on diversity of their indication(s) and years in use can be relatively compelling), and (2) their availability for immediate use in exposed and at-risk individuals (after demonstrating desired anti-coronaviral efficacy and obtaining regulatory approval). Here, we seek to review the efficacy, advantages, and disadvantages of repurposed virus-targeting antivirals and host-targeting agents, in particular serine protease-inhibiting anticoagulants, for protection against SARS-CoV-2.

2. Repurposing virus-targeting therapies against SARS-CoV-2

Conventional antiviral therapeutics function by targeting viral proteins that are critical for entry into the host cell or viral replication and subsequent release of virions from the infected host cell. Given that SARS-CoV-2, like other coronaviruses, is a single stranded RNA virus, nucleotide/nucleoside analogues are a class of antivirals that were rapidly repurposed for use against SARS-CoV-2 (Khan et al., 2021). Nucleoside/nucleotide analogues are purine and pyrimidine derivatives that function by targeting the viral RNA dependent RNA polymerase (RdRp), an evolutionarily conserved viral enzyme broadly utilized by RNA viruses for replication (Elfiky, 2021). These analogues can limit viral replication by covalently binding RdRp during RNA replication resulting in aberrant termination of the nascent RNA, development of lethal mutagenesis in the virus, and depletion of host nucleotides required for transcription (Pruijssers & Denison, 2019). Among the most notable analogues repurposed for SARS-CoV-2 are remdesivir, ribavirin, and favipiravir, which were originally approved for use against hepatitis C virus, respiratory syncytial virus, human immunodeficiency virus (HIV), Ebola, and influenza viruses (Mei & Tan, 2021). Several of these drugs exhibited high binding affinity for RdRp of SARS-CoV-2 and in vitro data showed their strong antiviral activity against the virus (Nguyen, Thai, Truong, & Li, 2020; Wang et al., 2020). However, despite initial findings on improved recovery in patients infected with SARS-CoV-2 when administering RdRp-inhibitors, additional clinical trials revealed that remdesivir and ribavirin have limited efficacy in reducing COVID-19 mortality (Beigel et al., 2020; World Health Organization Solidarity Trial Consortium, et al., 2021). Confounding results such as this indicate that more studies may be required to determine the true therapeutic value of these drugs for treatment of COVID-19.

In addition to RdRp, the virally encoded main protease of SARS-CoV-2 (M^{pro}) has become a prime viral target for therapeutic intervention (Sacco et al., 2020; Ullrich & Nitsche, 2020). M^{pro} is a conserved protease which, along with RdRp, is crucial for viral replication and transcription (Jin et al., 2020). Structural analyses of SARS-CoV-2 M^{pro} identified several well-established viral protease inhibitors, including anti-HIV drugs indinavir, darunavir and lopinavir, as potential inhibitors of the SARS-CoV-2-encoded protease (Komatsu et al., 2020; Sang, Tian, Meng, & Yang, 2020). However, despite their ability to inhibit SARS-CoV-2 M^{pro} in HEK-293 T cells in vitro, the effective dose of these drugs (in micromolar range) is likely to be high for in vivo administration, thereby limiting their clinical potential (Mahdi et al., 2020). In addition, the antiviral effect of some (e.g., lopinavir) was attributed to their cytotoxicity (Mahdi et al., 2020), and early clinical trials of lopinavir indicate minimal, if any, appreciable change in mortality with use of HIV protease inhibitors in COVID-19 patients (Cao et al., 2020; Hornby, 2020).

The disparate efficacy of M^{pro} inhibitors between SARS-CoV-2 and other viruses may stem from limited cross-species/strain interaction between the drugs and their target, which is a significant limitation of many virus-targeting therapeutics. The conventional antiviral drug discovery approach of identifying virus-specific druggable targets then optimizing interactions between drug and target protein inherently limits cross-protection (Dolgin, 2021). Moreover, use of conventional antivirals raises concerns with development of drug-resistance viral variants (Kumar et al., 2020; Kumar et al., 2021; Lampejo, 2020; Mason, Devincenzo, Toovey, Wu, & Whitley, 2018). Use of virus-targeting therapeutics excerpts a strong selective pressure on the virus, which coupled with the high rate of mutation associated with RNA virus replication can cause rapid shifts in viral drug targets – rendering therapeutics less effective (Dobrovolny & Beauchemin, 2017; Lampejo, 2020; Mason et al., 2018). The antiviral resistance can also arise and persist in the absence of drug selective pressures (Dobrovolny & Beauchemin, 2017), however, its pace and depth is unlikely to be similar to one triggered by widespread use of virus-targeting antivirals in general population. Notably, development of resistant virus strains was already well-characterized for many of these drugs prior to the COVID-19 outbreak (Beaucourt & Vignuzzi, 2014; Goldhill et al., 2018; Lv, Chu, & Wang, 2015; Mejer et al., 2020). Additionally, the discovery of silent RdRp mutations among SARS-CoV-2 mutational hotspots is particularly alarming and has heavy implications for the rise of drug resistance in the COVID-19 pandemic (Pachetti et al., 2020).

3. Host-targeting therapeutic strategies against SARS-CoV-2

With the rise of drug-resistant microbes, there has been a growing interest and drive to identify alternative strategies to conventional antiviral therapeutics. One promising avenue has been targeting the host.

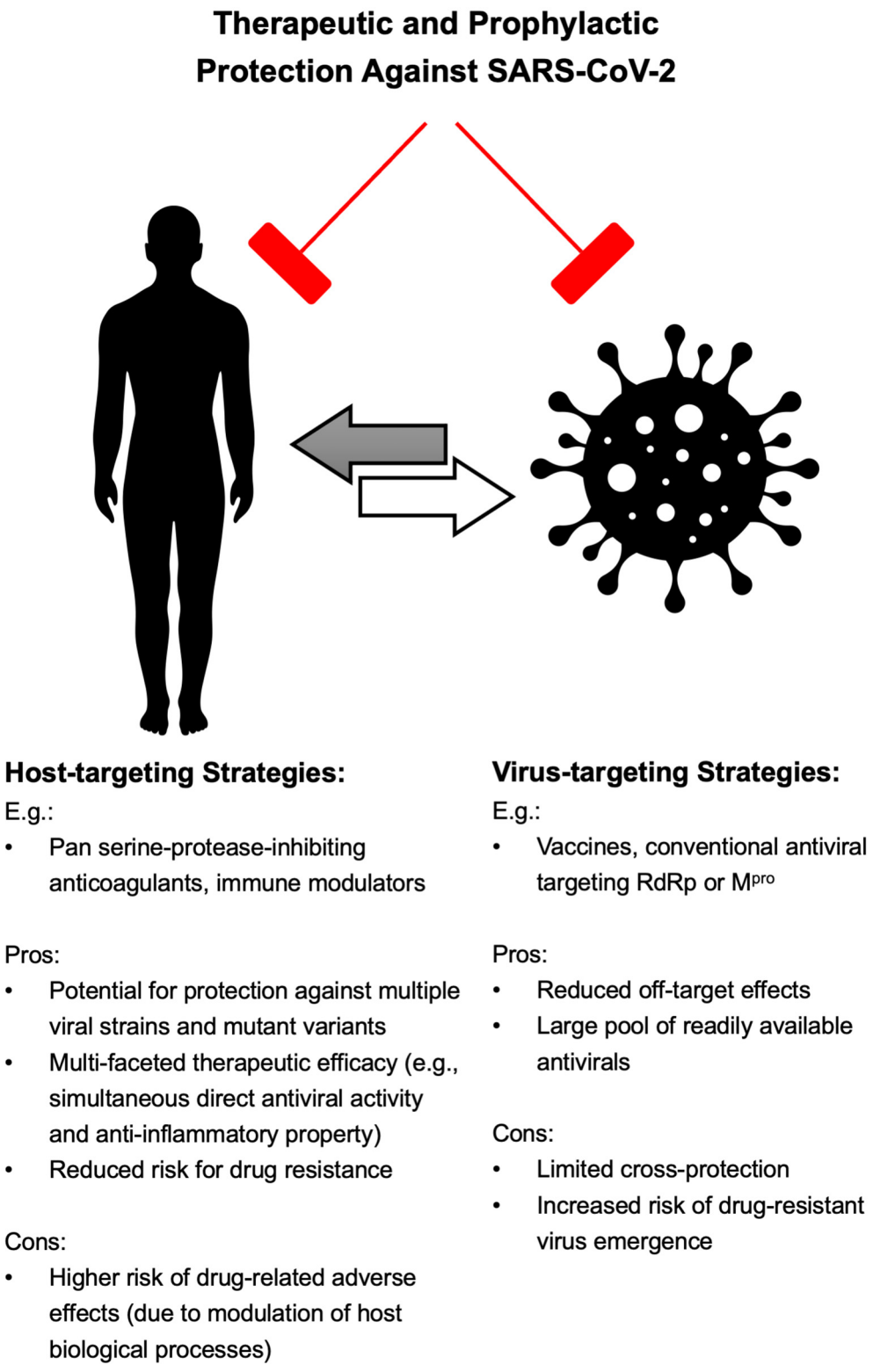

Fig. 1. Comparison of Host- vs. Virus-targeting Therapeutic and Prophylactic Strategies. Schematic of major approaches used for treatment of SARS-CoV-2 infection and their advantages and disadvantages. Information described here was found from the following citations (Dobrovolny & Beauchemin, 2017; Dolgin, 2021; Kumar et al., 2021; Niemeyer et al., 2021). The images of human and coronavirus were acquired from Shutterstock Images.

Unlike pathogen-targeting antivirals, host-based therapies do not inhibit virus infection by targeting viral components directly, but rather block host factors that are either key for viruses to be infective or lead to unregulated, often excessive, immune activation. We describe both strategies below.

3.1. Serine protease-inhibiting anticoagulants

Unlike virus-directed therapies, targeting of host factors that are essential for virus activation may potentially lead to cross-protection against emerging and re-emerging viruses, regardless of their strain,

and reduce risk of drug-resistant variants formation (Dolgin, 2021). Most of high- and low-pathogenic strains of CoVs converge on the same class of host molecular machinery for virus entry, replication, and release (Wong & Damania, 2021). For instance, SARS-CoV, MERS-CoV, and SARS-CoV-2 utilize host angiotensin-converting enzyme 2 (ACE2) as a receptor to attach to host cells (Flerlage, Boyd, Meliopoulos, Thomas, & Schultz-Cherry, 2021; Hatmal et al., 2020). By targeting host ACE2, rather than its ligand which is virus spike (S) protein – one of the key proteins covering surface envelop of coronaviruses, therapeutic candidates may elicit broad antiviral activity against most, if not all, CoVs that require ACE2 for docking. In fact, several naturally occurring compounds such as glycyrrhizin, nobiletin, and neohesperidin have been shown or are predicted to bind to and block ACE2 interaction with SARS-CoV-2 spike protein (Mei & Tan, 2021; Wu et al., 2020; Zhou & Huang, 2020). Of these, glycyrrhizin appears to be the most promising with proven antiviral activity and a clinical trial currently under way (NCT04487964) (van de Sand et al., 2021). ACE2 expression and activity play complex roles in coronavirus infection and lung injury. Coronaviruses, including SARS-CoV and SARS-CoV-2, have been shown to downregulate ACE2 expression following infection of target cells (Kai & Kai, 2020; Kuba et al., 2005). Both virus infection and downregulation of ACE2 can lead to increased levels of angiotensin II (Ang II), which is linked to lung injury, inflammation, and fibrosis through binding to receptors such as Ang II type 1 (AT1) (Kai & Kai, 2020; Rothlin et al., 2021; Wang et al., 2017). As such, use of Ang II receptor blockers (including losartan, valsartan, and telmisartan) has been proposed as a potential therapeutic intervention for COVID-19 (Kai & Kai, 2020; Rothlin et al., 2021). In support of this, early results from clinical trials suggest that Ang II receptor blockers do improve the outcome of COVID-19 patients (Rothlin et al., 2021). Telmisartan, for instance, has been shown to reduce the levels of c-reactive protein in patients' plasma and reduce the time until patient discharge (Duarte et al., 2021; Rothlin et al., 2021).

The CoV S proteins are the main determinant of entry into target cells since they possess both receptor binding and fusion functions. The S protein is a type I transmembrane protein located at the surface of the virion, with a large ectodomain and very short endodomain. The S protein ectodomain can be divided into two functional domains: the S1 domain which holds the receptor binding properties and the S2 domain which harbors the fusion machinery (Huang, Yang, Xu, Xu, & Liu, 2020). While binding to the host cell receptor (e.g., ACE2) is an essential first step in establishing infection, serine proteolytic activation of S protein is a critical step for the fusion function of the S to allow controlled release of the fusion peptide into target cellular membranes. Since CoVs do not code for a serine protease, they are completely dependent on host cell proteases for S activation to be infective. The key host proteases suggested to mediate activation of CoV S are Type II Transmembrane Serine Proteases (TTSPs; TMPRSS), in particular TMPRSS2 (Bertram et al., 2013; Glowacka et al., 2011; Hoffmann et al., 2020; Iwata-Yoshikawa et al., 2019; Matsuyama et al., 2020), although other surface bound proteases, such as furin, may also contribute to SARS-CoV-2 activation and entry (Tharappel, Samrat, Li, & Li, 2020; Wu et al., 2020). Bioinformatic analysis has revealed furin cleavage sites located in the S protein of SARS-CoV-2 (Wu, Zheng, et al., 2020). Computational screening uncovered a library of 4000 compounds with predicted interactions with furin, including the anti-parasitic drug diminazene (Wu, Zheng, et al., 2020). In vitro studies have shown that diminazene inhibits furin with half maximal inhibitory concentrations (IC_{50}) of 5.42 ± 0.11 μM (Wu, Zheng, et al., 2020). Given their importance in coronavirus infections, host proteases represent key druggable targets that can be exploited for therapeutic purposes (Tharappel et al., 2020).

Nafamostat mesylate is a low-molecular-weight synthetic broad-spectrum serine protease inhibitor that has been approved clinically as anticoagulant during hemodialysis and continuous renal replacement therapy as well as a treatment for pancreatitis in Japan and South Korea for over three decades (Choi et al., 2015; Pak et al., 1988; Park et al., 2011; Yoo et al., 2011). It also has inhibitory effects on plasmin activity and plasminogen activators (Ji, Wagener, Ness, & Zhao, 2021; Okajima, Uchiba, & Murakami, 1995; Uchiba, Okajima, Abe, Okabe, & Takatsuki, 1994). In a high-throughput evaluation of almost 1000 clinically approved drugs in vitro, Yamamoto et al. identified nafamostat as a potent inhibitor of MERS-CoV S protein-mediated membrane fusion using reporter cell lines. Moreover, the authors reported that camostat mesylate – an earlier analogue of nafamostat, was also effective in blocking MERS-CoV S protein activation, although at much lower potency (IC_{50} of 0.1 μM and 1 μM for nafamostat and camostat, respectively) (Yamamoto et al., 2016). With the emergence of SARS-CoV-2, several groups expanded upon these findings to demonstrate nafamostat and camostat are able to limit viral entry of SARS-CoV-2 using vesicular stomatitis virus (VSV) pseudotyped virus expressing SARS-CoV-2 spike protein in human Calu-3 lung cell line (Hoffmann et al., 2020; Hoffmann et al., 2021; Yamamoto et al., 2020). Most recently, Niemeyer et al. developed an in vitro SARS-CoV-2 infection model that utilizes polarized mucociliated primary human bronchiolar epithelia (Niemeyer et al., 2021). Using this model system and authentic viral particles (rather than pseudotyped virions) for infection, they observed that nafamostat inhibits apical virus shedding from infected epithelia reconstituted using cells derived from healthy non-smokers, smokers and subjects with chronic obstructive pulmonary disease (COPD; a COVID-19 comorbidity). Importantly, Niemeyer and colleagues revealed nafamostat has antiviral-independent anti-inflammatory properties by lowering homeostatic secretion of pro-inflammatory cytokines from human airway epithelia in the absence of viral challenge, and that this compound exhibits considerable antiviral efficacy against two seasonal human coronaviruses (hCoV-229E and hCoV-NL63) (Niemeyer et al., 2021). These findings illustrate cross-protective and multi-beneficial effects of (preclinical) application of nafamostat as a prime example of host-targeting strategy in treatment of SARS-CoV-2 and other coronaviral infections.

From a clinical perspective, two major concerns may arise when considering administration of nafamostat for COVID-19: its safety profile, and its undesired anticoagulatory and off-target effects. Specifically, one may argue nafamostat use may be associated with increased likelihood of cytotoxicity and disruption of homeostatic physiology, since it modulates host biological processes. While a valid concern, in vitro studies have revealed that localized delivery of nafamostat to well-differentiated (i.e., mucociliated) airway epithelial cells neither yields discernable cytotoxicity no induces cellular stress at its effective antiviral doses (Niemeyer et al., 2021). Additionally, transgenic animals lacking TTSPs mostly exhibit normal physiological functioning and survival rates comparable to wild-type controls. For instance, TMPRSS2$^{-/-}$ mice develop normally, survive to adulthood and have no defect in fertility or survival (Kim, Heinlein, Hackman, & Nelson, 2006; Sakai et al., 2014). Similarly, other in vivo studies with mice have shown that TMPRSS11A and TMPRSS11D are dispensable for development and health (Sales et al., 2011). Such observations imply that inhibition of host TTSPs may not be detrimental to host, particularly when treatment with a serine protease-blocking compound such as nafamostat is temporary. Furthermore, clinical data show nafamostat to be a safe and well-tolerated drug as an anticoagulant (Breining et al., 2021; Maruyama et al., 2011; Sawada et al., 2016). Thus, it is not surprising that as of July 2021 there are at least seven clinical trials using nafamostat for treatment of CVOID-19 underway (NCT04352400; NCT04418128; NCT04390594; NCT04628143; NCT04623021; NCT04473053; NCT04483960).

Avoiding undesired off-target effects of nafamostat requires thorough validation of TTSP family member(s) responsible for SARS-CoV-2 activation so that new derivatives of nafamostat with increased specificity to these target(s) can be developed. Currently, nafamostat similar to camostat is primarily thought to act through inhibition of TMPRSS2 (Hoffmann et al., 2021; Hoffmann, Kleine-Weber, et al., 2020; Niemeyer et al., 2021; Sonawane et al., 2021). While it is likely that

inhibition of TMPRSS2 by these drugs confers a degree of protection from SARS-CoV-2 infection, more research is needed to fully understand which TTSPs are essential for their antiviral (and anti-inflammatory) efficacy as several proteases other than TMPRSS2, such as TMPRSS4, TMPRSS11D, and TMPRSS13, have been shown to activate SARS-CoV-2 and related viruses (Kishimoto et al., 2021; Laporte et al., 2021; Zang et al., 2020). As such, comprehensive studies ideally using primary (not lines or immortalized) human (not animal)-derived cells are required where homeostatic surface protein expression of TTSPs in target tissues are well-characterized, and the protease expression can be modulated through knock-down systems (e.g., as performed via CRISPR by (Niemeyer et al., 2021)) (rather than overexpression) to identify serine proteases essential for SARS-CoV-2 activation. However, such efforts are partially hampered by absence of adequate bioreagents and poor understanding into biology of all TTSPs. For instance, not all members of TTSP family are well-characterized and limited specific, validated antibodies and chemical inhibitors are available to the scientific community.

Interestingly, early during the COVID-19 pandemic it was found that SARS-CoV-2 infection, particularly in severe cases, often becomes complicated with coagulopathies like stroke, thrombophilia, pulmonary embolisms, and disseminated intravascular coagulation (Chen et al., 2020; Connors & Levy, 2020; Merkler et al., 2020; Poissy et al., 2020; Tang et al., 2020). These pathologies are thought to arise from a mix of virus-associated factors including elevated inflammation, lung injury, and endothelial damage and dysfunction rather than direct coagulation via the virus (Carfora et al., 2021; Connors & Levy, 2020). Therefore, one may suggest another beneficial effect of nafamostat, besides its antiviral and anti-inflammatory properties, could be its ability to inhibit coagulation and plasmin activity (as well as plasminogen activators). In support of this, in a recent case report it has been shown that nafamostat and heparin combinatorial therapy has dramatic efficacy on a patient with COVID-19 pneumonia (Takahashi et al., 2021). Similarly, Jang et al. reported three cases of elderly patients with COVID-19 pneumonia whose disease progressed while taking antivirals and needing supplementary oxygen therapy but improved after receiving nafamostat (Jang & Rhee, 2020). Additionally, another small case study found that combination treatment of antiviral favipiravir and nafamostat may be effective (lowering mortality rate) for critically ill Covid-19 patients (Doi et al., 2020). Based on the initial limited number of clinical studies, it appears that hyperkalemia may be the major adverse event when treating COVID-19 patients with nafamostat (Okajima, Takahashi, Kaji, Ogawa, & Mouri, 2020). However, larger sample size and randomized controlled trials are required to detect adverse effects of nafamostat in COVID-19 more accurately and identify optimal dose and route(s) of administration (e.g., inhaled vs. oral vs. systemic). Moreover, timing (Takahashi et al., 2021) of nafamostat treatment needs to be identified (1) as this drug can theoretically be applied prophylactically for preemptive protection (in addition to its therapeutic efficacy), and (2) to minimize unnecessary prolonged delivery and/or undesired drug effects.

3.2. Immune modulators

Immune modulators represent another class of host-targeting therapeutics currently being applied in treatment of COVID-19, which primarily focus on either boosting antiviral immunity or dampening exaggerated excessive immune activation such as cytokine storm (Rizk et al., 2020; Song, Li, Xie, Hou, & You, 2020). Interferon alpha-2a and -2b are immune modulators which have been used to treat both hepatitis B and C infections as well as SARS-CoV and MERS-CoV (Maughan & Ogbuagu, 2018; Woo, Kwok, & Ahmed, 2017; Zeng et al., 2020). Interferon alpha has been shown to limit SARS-CoV-2 replication and early reports indicate its use significantly improved clinical outcomes in COVID-19 patients (Lokugamage et al., 2020; Pandit et al., 2021). Colchicine, an anti-inflammatory approved for treatment of gout in 2009, has also received much attention as a COVID-19 therapeutic agent (Reyes et al., 2020; Vitiello & Ferrara, 2021). Early reports suggest that colchicine treatment of COVID-19 patients results in reduced clinical deterioration and mortality, this is despite observing limited reduction in inflammatory biomarkers (Deftereos et al., 2020; Vrachatis et al., 2021).

Immune modulation may also be achieved through pharmacological activation of host factors with direct and indirect anti-inflammatory activities, such as nuclear factor erythroid 2 p45-related factor 2 (Nrf2). Nrf2 is a transcription factor which heterodimerizes with numerous other factors to regulate antioxidant response elements, redox homeostasis, damage repair, and cellular redox homeostasis and can control inflammation by repressing interleukin 6 (IL6) and interleukin 1 beta (IL1B) expression (Cuadrado et al., 2020; Kobayashi et al., 2016). Apart from their anti-inflammatory properties, some Nrf2 activators, such as bardoxolone and bardoxolone methyl, have been shown to directly limit SARS-CoV-2 replication by inhibition of its main protease (Sun et al., 2021). Similar in principle to the anti-inflammatory effects of Nrf2 activation, direct IL6 antagonists, including monoclonal antibodies tocilizumab and sarilumab are predicted to have therapeutic value in treating COVID-19 and cytokine storm associated with virus infection (Castelnovo et al., 2021; Lu, Chen, Lee, & Chang, 2020; Zhang, Zhong, Pan, & Dong, 2020). Clinical studies suggest that for patients with medium-to-severe forms of COVID-19 pneumonia, early intervention with tocilizumab and sarilumab is indeed associated with positive therapeutic outcomes including increased survival; however, treatment must be administered very early during infection (Castelnovo et al., 2021; Investigators et al., 2021).

Besides conventional drugs, cell-based therapeutic interventions utilizing mesenchymal stem cell (MSC) delivery to patients with COVID-19 represents a unique immune-modulating treatment modality. MSCs are capable of suppressing activated immune cells though direct cell-cell contacts via programmed death-ligand 1 (PD-L1) and Fas ligand (FasL), and can reduce proinflammatory cytokine and chemokines in the lung during influenza infection (Kavianpour, Saleh, & Verdi, 2020). Additionally, MSCs are known to promote repair of lung injury during COPD, asthma, pneumonia, and idiopathic pulmonary fibrosis (Harrell et al., 2019). In the context of COVID-19, multiple clinical trials are underway and initial findings indicate that MSC treatment can reduce lung injury and improve patient outcome (Shi et al., 2021).

Finally, hormones such as vitamin D can play key a role in regulating host immune response during virus infections. At the airway epithelium, vitamin D promotes protection from pathogens both through production of antimicrobial β-defensins and cathelicidin and maintenance of epithelial junctions (Banerjee et al., 2021). Moreover, vitamin D3 has been shown to limit respiratory virus replication and alter expression of interleukin 8 and interferons (Banerjee et al., 2021; Telcian et al., 2017). In the context of COVID-19, lower levels of vitamin D3 have been associated with more severe disease including mortality and proinflammatory cytokines in the serum of patients (Banerjee et al., 2021). Further studies found that vitamin D3 supplementation reduced the disease severity, diminished admittance to the intensive care, and lowered mortality rates among patients with COVID-19 (Banerjee et al., 2021; Entrenas Castillo et al., 2020; Ling et al., 2020). Altogether, immune modulation exhibits great potential in treating COVID-19, yet truly effective application of this class of drugs, cell therapies and vitamin supplements requires a much clearer understanding of the disease pathogenesis and particularly disease kinetics as timing, dosing, and patient selection are vital to therapeutic efficacy (Snow, Singer, & Arulkumaran, 2020).

4. Conclusion and outlook

The rise of SARS-CoV-2 and consequent COVID-19 pandemic has placed the global health communities in a precarious position. We are in dire need of effective therapies to treat existing and emerging strains

of SARS-CoV-2; yet we lack the time needed to develop new targeted drugs. Thus, repurposing therapeutics that are already approved for other indications may provide an untapped resource for treatment of SARS-CoV-2 infection and associated pathologies. Host-targeting therapeutic strategies, and in particular serine protease-inhibiting anticoagulants such as nafamostat, provide a strong alternative to conventional drugs. Specifically, nafamostat exerts antiviral, anti-inflammatory and anticoagulatory effects which are complementary and may be highly beneficial to COVID-19 patients. Importantly, nafamostat, by acting on host molecular machinery that SARS-CoV-2 and several seasonal human coronaviruses need to hijack to their advantage to cause infection, offers cross-protection against multiple CoV strains and could be deployed rapidly as new viral variants emerge. However, moving forward more clinical insight into optimal dose and route(s) of administration, prophylactic vs. therapeutic use, timing of application and adverse effects of nafamostat are needed. Randomized controlled trials with large number of participants would provide valuable information on this end. In addition, rigorous comprehensive preclinical studies are needed to thoroughly validate exact target of SARS-CoV-2, its mutant variants and other CoVs in primary human cells for new compound development for future. Such approach would allow minimizing adverse and off-target effects of nafamostat. Lastly, while we present a strong case for use of host-targeting therapeutics over traditional antivirals for treatment of COVID-19 and other coronaviral infections, we do not propose that this should entirely replace virus-targeting drugs. Rather, these two distinct strategies complement one another to help mitigate the burden of current and future CoV outbreaks.

Author contributions

B.F.N., and K.H.B. drafted and critically revised the manuscript.

Declaration of Competing Interest

K.H.B. is founder and holds equity in Pneumax, LLC. B.F.N. declares no conflict of interest.

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