TMPRSS2 inhibitors, Bromhexine, Aprotinin, Camostat and Nafamostat as potential treatments for COVID-19.

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Abstract

In December 2019 SARS-CoV-2 emerged in Wuhan, China, leading to a pandemic in March 2020. Although clinical presentation of COVID-19 ranges from no symptoms to acute respiratory and multiorgan failure, the vast majority of the patients only experience flu-like symptoms. However because of highly contagious nature of the disease, overall COVID-19 is considered a global health burden. Since ACE2, TMPRSS2 expressing cells are the potential hosts for the virus, inhibiting TMPRSS2 by clinically proven protease inhibitors Bromhexine, Aprotinin, Camostat and Nafamostat are suggested as potential treatments for COVID-19.

Keywords

SARS-CoV-2, COVID-19, Pathophysiology, Pharmacology, TMPRSS2 inhibitor, Bromhexine, Aprotinin, Camostat, Nafamostat

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Highlights

–SARS-CoV-2 infects ACE2, TMPRSS2 expressing cells

–Inhibiting TMPRSS2 hinders infection of host cells with SARS-CoV-2

–Bromhexine, Aprotinin, Camostat and Nafamostat inhibit TMPRSS2

–Bromhexine, Aprotinin, Camostat and Nafamostat may contribute to treatment of COVID-19

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Introduction

Coronavirus disease 2019 (COVID-19) is an illness that is caused by Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) (Zheng et al.,2020; Wu and McGoogan 2020). In December 2019 SARS-CoV-2 emerged in Wuhan, China and thereafter spread throughout the world and in March 11, 2020, World Health Organization declared COVID-19 a pandemic (Zheng et al., 2020; Wu and McGoogan 2020).

Currently, COVID-19 seems to transmit from person to person via respiratory droplets (Wu and McGoogan 2020). Additionally, as in cases of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), SARS-CoV-2 is also possibly transmitted via fecal-oral route (Yeo et al., 2020). Common signs and symptoms of COVID-19 include fever, sore throat, cough, malaise, headache, nausea, vomiting, diarrhea and breathlessness (Zheng et al., 2020; Wu and McGoogan 2020; WHO, 2020b; Wu and McGoogan 2020; Chan et al., 2020). The spectrum of clinical symptoms of COVID-19 varies from mild, (with no or mild pneumonia), to severe and critical with respiratory failure and multiorgan dysfunction (Wu and McGoogan, 2020; WHO, 2020b; Yeo et al., 2020).

Because of highly contagious nature of SARS-CoV-2 (WHO, 2020b; Yeo et al., 2020) and potential mortality (Wu and McGoogan 2020; WHO, 2020b; Thienemann et al., 2020) it is very important to find treatments to prevent and/or perhaps control the disease (WHO, 2020b). This article is dedicated to theoretical evaluation of possible effects of some already available medications, including Bromhexine, Aprotinin, Camostat and Nafamostat, on pathogenesis of SARS-CoV-2.

Pathophysiology of COVID-19

SARS-CoV-2 engages with Angiotensin Converting Enzyme-2(ACE-2) as entry receptor and uses serine protease TMPRSS2 (Serine protease 10) for S protein priming and fusion of viral and cellular membranes, to enter the host cell. (Hoffmann et al., 2020a). ACE-2 converts Angiotensin-2 to angiotensin-(1-7) (Ang-(1-7)) which stimulates MAS oncogene. This ACE2- Ang-(1–7)-Mas axis, elicits vasodilatory, anti-thrombotic, anti-oxidative, anti-inflammatory, anti-apoptotic, anti-proliferative, anti-hypertrophic, anti-fibrotic, natriuretic and diuretic effects and also enhances endothelial function (Iwai and Horiuchi, 2009). ACE-2 receptors are expressed on the endothelia and smooth muscle cells of nearly all organs, as well as alveolar epithelial cells, myocytes, renal glomerular and tubular cells, intestinal

mucosal cells, brain neurons, and testicles (Hamming et al., 2020; Crackower et al., 2002; Donoghue et al., 2000; Tikellis et al., 2003; Tipnis et al., 2000; Williams and Scholey, 2018; Xu et al., 2011).

TMPRSS2 is a mostly membrane-bound serine-protease that promotes metastasis of prostate cancer, increases expression of some matrix metalloproteinases and in hepatocytes it contributes to tyrosin kinase signalling pathways (Lucas et al., 2014). However, the exact physiological role of TMPRSS2 has not yet been fully explored (Kim et al., 2006, Lucas et al., 2014). TMPRSS2 is expressed on endothelial cells, epithelia of the gastrointestinal, urogenital and respiratory tract, as well as alveolar epithelial cells, myocytes, hepatocytes, salivary glands and prostate (Aimes et al., 2003; Paoloni et al., 1997; Glowacka et al., 2011; Vaarala et al., 2001). According to simultaneous expression of ACE-2 and TMPRSS2 in alveolar epithelial and endothelial cells, theoretically these cells could be the possible primary host cells for SARS-CoV-2.

There are two different types of alveolar epithelial cells in the alvoli. Type I cells, which make up 90% of the alveolar epithelium, and Type II cells which serve as stem cell of the alveoli, they proliferate and give rise to all alveolar epithelial cells (Fehrenbach, 2001; Famous et al., 2017). Type II alveolar epithelial cells also synthesise, secrete, and recycle all components of the surfactant and contribute to alveolar repair (Fehrenbach, 2001; Famous et al., 2017). Destruction of Type II cells, decreases intracellular stores, secretion and recycling of surfactant and thereby impairs alveolar function and decreases lung compliance (Fehrenbach, 2001; Famous et al., 2017). Compared to Type-I alveolar epithelial cells, Type II cells express more ACE2 on their surface, which favours implantation of SARS-CoV-2 (McGonagle et al., 2020; Hamming et al., 2004; Fehtenbach, 2001). As SARS-CoV-2 implants and replicates in Type II cells, as a reparative reaction, these cells start to proliferate (McGonagle et al., 2020; Chan et al., 2020; Fehrenbach, 2001; Famous et al., 2017). As number of Type II cells increases, the virus infects them and replicates to a higher extent, leading to high viral load and rapidly progressing fulminant pneumonia (Chan et al., 2020; Fehrenbach, 2001; Famous et al., 2017) (Figure 1, 1-6).

At the time of alveolar injuriy, Type II cells express Inter-Cellular Adhesion Molecule-1(ICAM-1) and Vascular Cell Adhesion Molecule-1 **(**VCAM-1) and release the chemoattractants that attract monocytes and macrophages to the place, and these immune cells release several inflammatory cytokines such as TNF-alpha, which prompts Type II cells to stimulate expression of P-selectin in perialveolar capillary network (Kuebler et al., 2000; Rosseau et al., 2000). This further increases local recruitment of immune cells and causes a

rapid and extensive inflammatory response (Famous et al., 2017; Kuebler et al., 2000; Rosseau et al., 2000). As a consequence of inflammation and vasculoendothelial damage, TMPRSS2 is expressed on endothelial surface of capillaries to a higher extent (Aimes et al., 2003; Famous et al., 2017). Based on this scenario, it is concluded that subsequent expression of higher amounts of ACE2 and TMPRSS2 on endothelial surface of inflamed perialveolar capillary network (Aimes et al., 2003) and Type II cells (Fehrenbach, 2001), may contribute to high viral burden. This sequence of events could lead to ARDS and to dissemination of SARS-CoV-2 throughout the circulation, causing viral sepsis, massive inflammatory response, cardiovascular complications and multiorgan failure, which are the major leading causes of mortality and morbidity of COVID-19 patients (Zheng et al., 2000; McGonagle et al., 2020; Wu and McGoogan, 2020; WHO, 2020b; Chan et al., 2020; Thienemann et al., 2020) (Figure 1, 7-15).

A Preventive and Therapeutic approache to COVID-19

Considering the above scenario, it is reasonable to hypothesize that by inhibiting implantation of SARS-CoV-2 in the Type II cells and perialveolar capillary network, we may hinder high viral burden and further septicaemia, massive inflammatory response and multiorgan failure. In this regard, one may think about decreasing expression, release and/or activity of ACE2 and TMPRSS2.

However, it has been shown that attenuation of ACE2 aggravates oxidative stress and inflammation and increases permeability of the alveolar-capillary barrier, reinforces sodium and water retention, worsens pulmonary edema and accelerates respiratory deterioration (Gopallawa and Uhal, 2014; Simoes e Silva et al., 2013).

In contrast, a great body of evidence demonstrates that decreasing expression and/or activity of TMPRSS2 is a relatively safe and an effective method for treatment of viral infections that are caused by viruses (such as MERS-CoV, SARS-CoV, SARS-CoV-2, several H1N1 subtypes of influenza A viruses and Asian H7N9 influenza virus) that utilize TMPRSS2 for implantation in host cells (Hoffmann et al., 2020a; Hoffmann et al., 2020b; Sanders et al., 2020; Bestle et al., 2020; Bittmann et al., 2020; Sonawane et al. 2020; Shen et al., 2017; Glowacka et al., 2011; Hamming et al., 2004). These studies have shown that virulence of the above-mentioned viruses depend on serine protease activity of TMPRSS2 (Hoffmann et al., 2020a; Hoffmann et al., 2020b; Sanders et al., 2020; Bestle et al., 2020; Bittmann et al., 2020; Sonawane et al. 2020; Shen et al., 2017; Glowacka et al., 2011; Hamming et al., 2004). In

these viral infections reducing the level of activity of TMPRSS2, is followed by a significant decrease in the rate of implantation, local replication, dissemination and secondary replication of the viruses (Hoffmann et al., 2020a; Hoffmann et al., 2020b ; Shen et al., 2017; Glowacka et al., 2011). Since SARS-CoV-2 is also one of the viruses that uses TMPRSS2 for implantation, it is suggested that inactivating TMPRSS2 with clinically proven TMPRSS2 inhibtors can contribute to treatment of COVID-19 (Hoffmann et al., 2020a; Sanders et al., 2020; Bestle et al., 2020; Bittmann et al., 2020; Sonawane et al. 2020). Camostat, Nafamostat and Aprotinin are some of these TMPRSS2 inhibitors and are shown to reduce the rate of infection of lung cell line (Calu-3) by SARS-CoV-2, rendering these medications as potential treatments for COVID-19(Hoffmann et al., 2020a; Hoffmann et al., 2020b; Yamamoto et al., 2020; Bestle et al., 2020; Sonawane et al. 2020).

TMPRSS2-inhibitors

Bromhexine and its active metabolite Ambroxol: Bromhexine is a plant derived, mucolytic medication with a wide therapeutic window and few reported adverse effects in adults (Zanasi et al., 2017). Bromhexine has been used to decrease viscosity of pulmonary mucosal secretions, aiding mucocilliary clearance of the lungs and to supress cough (Irwin and Curley, 1991; Zanasi et al., 2017).

Bromhexine hydrochloride is shown to inhibit protease activity of TMPRSS2 in metastatic prostate cancer of mice and accordingly it is hypothesized that it could also be used for treatment of both influenza and coronavirus infections (Bestle et al., 2020; Sonawane et al., 2020; Shen et al., 2017; Lucas et al., 2014). Indeed, Bromhexine interacts with Gln438 and other residues present in the active site pocket of TMPRSS2 and inhibits TMPRSS2 mediated activation of a zymogen precursor of tissue plasminogen activator (Sonawane et al., 2020; Lucas et al., 2014). In body, Bromhexine is largely converted into Ambroxol (Malerba and Ragnoli, 2008). As the main active metabolite of Bromhexine, Ambroxol enhances production of surfactant and has antioxidative, anti-inflammatory properties (Malerba and Ragnoli, 2008; Seifart et al., 2005). It is shown that Ambroxol attenuates chemotaxis of immune cells to the site inflammation, decreases secretion of Interferon-γ (IFNγ), TNF-alpha, IL-1β, IL-6, IL-8 as well as arachidonic acide metabolites, and reduce lipid peroxidiation of inflamed tissues (Beeh et al., 2008; Malerba and Ragnoli, 2008). In addition high dose Ambroxol improves course and outcomes of ARDS (Wu et al., 2014).

Bromhexine and Ambroxol are available in form of syrups, tablets and injectable solutions (Zanasi et al., 2017; Cazan et al., 2018; Kantar et al., 2020). In addition a Lung-targeting Injectable Microsphere Bromhexine Hydrochloride Solution is also available and is shown to deliver a significantly higher concentration of the medication into the lungs (Liu et al., 2012). To our knowledge, Bromhexine is a safe medication and reported adverse effects of the medication in adults and children six years of age and over include nausea, rash, vomiting, diarrhea that are mild and rare (Zanasi et al., 2017; WHO, 2015). However there are some reports about rare but serious allergic reactions associated with the use of Bromhexine, in children younger than six years of age (WHO, 2015). Thereby Bromhexine should only be used in adults and children six years of age and over (WHO, 2015). In order to boost antioxidative and anti-inflammatory properties of Bromhexine, Ambroxol can be added to the medication (Deretic and Timmins, 2019). Clinical evidences reveal that Ambroxol is a relatively safe, efficacious and well tolerated medication, with a well-balanced and favourable benefit-risk profile (Cazan et al., 2018; Kantar et al., 2020). Thereby Bromhexine (plus Ambroxol) can be considered as a candidate for primary prevention and/or treatment of COVID-19.

Aprotinin: Aprotinin, the basic trypsin inhibitor of bovine pancreas, is a 58 amino acid broad spectrum serine protease inhibitor (Bestle et al., 2020). Through inhibition of killikrein, thrombin and plasmin, it attenuates inflammatory, coagulation and fibrinolytic pathways (Bestle et al., 2020; Westaby, 1993). Under the trade name Trasylol, injectable Aprotinin has been used to reduce risk of bleeding during major surgeries (Westaby, 1993). In Russia inhaled Aprotinin is approved to treat mild-to-moderate forms of influenza and parainfluenza in humans (Ovcharenko and Zhirnov, 1994). Aprotinin inhibits serine protease activity of TMPRSS2 in a dose-dependent manner and when it is used in aerosolized form, it is shown to reduce the rate of mortality of influenza and parainfluenza by more than 50% in mice (Böttcher et al., 2009; Ovcharenko and Zhirnov, 1994). Aprotinin is also shown to inhibit implantation of SARS-CoV-2 in Clu-3 lung cells in a dose dependent manner (Bestle et al., 2020). However, atrial fibrillation, fever and nausea are amongst the adverse effects of the medication (Westaby, 1993; Cosgrove et al., 1992). Accordingly further studies could evaluate possible therapeutic effects of inhaled and injectable Aprotinin on the course and outcomes of COVID-19.

Camostat and Nafamostat: Camostat, an oral serine protease inhibitor, is used for treatment of chronic pancreatitis and postoperative reflux esophagitis in some countries (Yamamoto et al., 2016). Camostat restrains Trypsin and thereby inhibits Plasmin, Kallikrein and Thrombin

(Ramsey et al., 2019). It is also shown to reduce the rate of infection of Calu-3 lung cells by SARS-CoV-2 (Hoffmann et al., 2020a).

Reported adverse effects of Camostat such as pruritus, increased thirst and appetite, and light headedness are usually tolerable (Ramsey et al., 2019).

Nafamostat is another broad spectrum synthetic serine protease inhibitor with anticoagulant and anti-inflammatory properties that inhibits prothrombin, coagulation factor X, coagulation factor XII, Trypsin-1, Kallikrein-1 and ICAM-1 (Kim et al., 2016). Currently in Japan parental Nafamostat is used for patients undergoing continuous renal replacement therapy to prevent coagulation and clot formation during extracorporeal circulation (Miyatake et al., 2017). Nafamostat, is an antioxidant, reduces levels of interleukin (IL)-6 and IL-8, inhibits TNF-alpha-induced oxidative stress, and decreases the rate of endothelial cell apoptosis (Kang et al., 2015). Both Camostat and Nafamostat interact with Asp435, Ser441 and His296 residues of TMPRSS2 and inhibit its action (Sonawane et al. 2020). They are shown to significantly reduce the rate of implantation of MERS-CoV and SARS-CoV-2 in Calu-3 lung cells (Hoffmann et al., 2020a ; Hoffmann et al., 2020b; Yamamoto et al., 2020; Yamamoto et al. 2016). However, Nafamostat does not seem to be as safe as Camostat and it is reported to have adverse effects such as agranulocytosis, hyperkalemia, anaphylaxis, and cardiac arrest (Muto et al., 1994).

Accordingly, future studies could evaluate possible therapeutic effects of Camostat on course and outcomes of COVID-19, while Nafamostat is better to be reserved for severe and critical cases of COVID-19, who suffer from severe inflammatory response and cytokine storm. Furthermore, WHO guidelines recommend extracorporeal membrane oxygenation (ECMO) to treat severe and critical COVID-19 patients who usually suffer from refractory hypoxemia and ARDS (WHO, 2020a). Nafamostat alleviates ARDS and also prevents coagulation and clot formation during extracorporeal circulation in these cases (Han et al., 2011; Uchiba et al., 1997). Thereby Nafamostat may be of value in COVID-19 patients who suffer from ARDS and/or need ECMO.

Conclusion

COVID-19 pandemic is a global health burden. Many clinical trials have taken place and therapeutic efficacy of many medications such as Tocilizumab, Sarilumab, Arbidol, Chloroquine, Hydroxychloroquine, Lopinavir, Darunavir, Ribavirin, Remdesivir and Favipiravir are being evaluated but since yet, none has been approved as the licensed

treatment for the disease (Sanders et al., 2020). Theoretically, TMPRSS2 inhibitors such as Bromhexine, Aprotinin, Nafamostat and Camostat, may reduce primary transmission, viral load, spread, dissemination and secondary replication of SARS-CoV-2 (Sanders et al., 2020; Bestle et al., 2020; Bittmann et al., 2020; Sonawane et al. 2020). Aprotinin,Camostat and Nafamostat are already shown to reduce the rate of implantation of the SARS-CoV-2 in Calu-3 lung cells (Hoffmann et al., 2020a; Hoffmann et al., 2020b; Yamamoto et al., 2020; Bestle et al., 2020; Sonawane et al. 2020).

Currently, several clinical trials are being conducted to evaluate possible therapeutic effects of Camostat (NCT04321096, NCT04338906, NCT04353284), Nafamostat (NCT04352400) and Bromhexine (NCT04273763, NCT04340349, IRCT20200317046797N4) on the course and outcomes of COVID-19.

Since Bromhexin facilitates mucocilliary clearance of lungs and reduces intensity and frequency of coughing, we have hypothesized that it may reduce the rate of primary and secondary transmission of COVID-19. Since it is metabolized into Ambroxol, which is shown to improve course and outcomes of ARDS (Wu et al., 2014), we have hypothesized that Bromhexin may improve outcomes of COVID-19. Ambroxol also decreases secretion of IFNγ, TNF-alpha, IL-1β, IL-6, IL-8, which are all shown to be up-regulated during COVID-19 (Wan et al., 2020; McGonagle et al., 2020; Wu et al., 2014; Beeh et al., 2008). Thereby we have hypothesized that addition of Ambroxol to Bromhexine may increase possible therapeutic effects of Bromhexine on course of COVID-19 (Figure 1, A-H).

For future studies, oral Camostat and/or Bromhexine (plus Ambroxol) as well as inhaled Aprotinin could be considered for primary prevention and/or treatment of mild and moderate cases of the disease. Whereas Parental Nafamostat, Aprotinin and/or Lung-targeting Injectable Bromhexine are better to be reserved for sever and critical cases of COVID-19. Nafamostat could be used as an anticoagulant in those who need ECMO (Han et al., 2011). Nafamostat and/or Ambroxol improve course and outcomes of ARDS (Wu et al., 2014; Uchiba et al., 1997) and we have hypothesized that, addition of Ambroxol to current therapeutic regiments of COVID-19 may facilitate treatment of the disease. We have also hypothesized that Ambroxol and Nafamostat may elicit accumulative anti-inflammatory effect.

In summary, SARS-CoV-2 utilizes ACE2 and TMPRSS2 to infect primary host cells and thereafter it may rapidly disseminate through the lungs and other parts of the body, causing Acute Respiratory Distress Syndrome (ARDS), endocarditis, myocarditis and multi-organ failure. Accordingly TMPRSS2 inhibitors, Bromhexine, Aprotinin, Camostat and Nafamostat

are suggested as potential treatments for COVID-19 and *in vitro*, Aprotinin, Camostat and Nafamostat are shown to inhibit SARS-CoV-2.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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References

- Aimes, R. T., Zijlstra, A., Hooper, J. D., Ogbourne, S. M., Sit, M. L., Fuchs, S., ... & Antalis, T. M. (2003). Endothelial cell serine proteases expressed during vascular morphogenesis and angiogenesis. *Thrombosis and haemostasis*, *89*(03), 561-572. doi: 10.1095/biolreprod.109.076273

- Beeh, K. M., Beier, J., Esperester, A., & Paul, L. D. (2008). Antiinflammatory properties of ambroxol. *Eur J Med Res*, *13*(12), 557-562. doi: 10.1095/biolreprod.109.076273

- Bestle, D., Heindl, M. R., Limburg, H., Pilgram, O., Moulton, H., Stein, D. A., ... & Becker, S. (2020). TMPRSS2 and furin are both essential for proteolytic activation and spread of SARS-CoV-2 in human airway epithelial cells and provide promising drug targets. *bioRxiv*.

- Bittmann, S., Weissenstein, A., Moschüring-Alieva, E., Bittmann, L., Luchter, E., & Villalon, G. (2020). The Role of TMPRSS2 and TMPRSS2-Inhibitors in Cell Entry Mechanism of COVID-19. *J Regen Biol Med*, *2*(3), 1-3.

- Böttcher, E., Freuer, C., Steinmetzer, T., Klenk, H. D., & Garten, W. (2009). MDCK cells that express proteases TMPRSS2 and HAT provide a cell system to propagate influenza viruses in the absence of trypsin and to study cleavage of HA and its inhibition. *Vaccine*, *27*(45), 6324-6329. doi: 10.1016/j.vaccine.2009.03.029.

- Cazan, D., Klimek, L., Sperl, A., Plomer, M., & Kölsch, S. (2018). Safety of ambroxol in the treatment of airway diseases in adult patients. *Expert opinion on drug safety*, *17*(12), 1211-1224. doi: 10.1080/14740338.2018.1533954.

- Chan, J. F. W., Yuan, S., Kok, K. H., To, K. K. W., Chu, H., Yang, J., ... & Tsoi, H. W. (2020). A familial cluster of pneumonia associated with the 2019 novel

coronavirus indicating person-to-person transmission: a study of a family cluster. *The Lancet*, *395*(10223), 514-523. doi: 10.1016/S0140-6736(20)30154-9.

- Cosgrove, D. M., Heric, B., Lytle, B. W., Taylor, P. C., Novoa, R., Golding, L. A., ... & Loop, F. D. (1992). Aprotinin therapy for reoperative myocardial revascularization: a placebo-controlled study. *The Annals of thoracic surgery*, *54*(6), 1031-1038.

- Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., ... & Scholey, J. (2002). Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*, *417*(6891), 822-828.

- Deretic, V., & Timmins, G. S. (2019). Enhancement of lung levels of antibiotics by ambroxol and bromhexine. *Expert opinion on drug metabolism & toxicology*, *15*(3), 213-218. doi: 10.1080/17425255.2019.1578748.

- Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., ... & Breitbart, R. E. (2000). A novel angiotensin-converting enzyme–related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation research*, *87*(5), e1-e9.

- Famous, K. R., Delucchi, K., Ware, L. B., Kangelaris, K. N., Liu, K. D., Thompson, B. T., & Calfee, C. S. (2017). Acute respiratory distress syndrome subphenotypes respond differently to randomized fluid management strategy. *American journal of respiratory and critical care medicine*, *195*(3), 331-338. doi: 10.1164/rccm.201603-0645OC.

- Fehrenbach, H. (2001). Alveolar epithelial type II cell: defender of the alveolus revisited. *Respiratory research*, *2*(1), 33. DOI: 10.1186/rr36

- Glowacka, I., Bertram, S., Müller, M. A., Allen, P., Soilleux, E., Pfefferle, S., ... & Niemeyer, D. (2011). Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by

the humoral immune response. *Journal of virology*, *85*(9), 4122-4134. doi: 10.1128/JVI.02232-10.

- Gopallawa, I., & Uhal, B. D. (2014). Molecular and cellular mechanisms of the inhibitory effects of ACE-2/ANG1-7/Mas axis on lung injury. *Current topics in pharmacology*, *18*(1), 71.
- Han, S. J., Kim, H. S., Kim, K. I., Whang, S. M., Hong, K. S., Lee, W. K., & Lee, S. H. (2011). Use of nafamostat mesilate as an anticoagulant during extracorporeal membrane oxygenation. *Journal of Korean Medical Science*, *26*(7), 945-950. doi: 10.3346/jkms.2011.26.7.945

- Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *203*(2), 631-637. DOI: 10.1002/path.1570

- Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., ... & Müller, M. A. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. DOI: 10.1016/j.cell.2020.02.052

- Hoffmann, M., Schroeder, S., Kleine-Weber, H., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). Nafamostat mesylate blocks activation of SARS-CoV-2: New treatment option for COVID-19. *Antimicrobial Agents and Chemotherapy*. doi: 10.1128/AAC.00754-20.

- Irwin, R. S., & Curley, F. J. (1991). The treatment of cough: a comprehensive review. *Chest*, *99*(6), 1477-1485. DOI: 10.1378/chest.99.6.1477

- Iwai, M., & Horiuchi, M. (2009). Devil and angel in the renin–angiotensin system: ACE–angiotensin II–AT 1 receptor axis vs. ACE2–angiotensin-(1–7)–Mas receptor axis. *Hypertension Research*, *32*(7), 533-536. DOI: 10.1038/hr.2009.74

- Kang, M. W., Song, H. J., Kang, S. K., Kim, Y., Jung, S. B., Jee, S., ... & Kim, C. S. (2015). Nafamostat mesilate inhibits TNF-α-Induced vascular endothelial cell dysfunction by inhibiting reactive oxygen species production. *The Korean Journal of Physiology & Pharmacology*, *19*(3), 229-234. doi: 10.4196/kjpp.2015.19.3.229

- Kantar, A., Klimek, L., Cazan, D., Sperl, A., Sent, U., & Mesquita, M. (2020). Ambroxol for the treatment of children with acute and chronic respiratory diseases: an overview of efficacy and safety. *Multidisciplinary Respiratory Medicine*, *15*. doi: 10.4081/mrm.2020.511

- Kim, H. S., Lee, K. E., Oh, J. H., Jung, C. S., Choi, D., Kim, Y., ... & Noh, H. (2016). Cardiac arrest caused by nafamostat mesilate. *Kidney research and clinical practice*, *35*(3), 187-189. https://doi.org/10.1016/j.krcp.2015.10.003

- Kim, T.S., Heinlein, C., Hackman, R.C., Nelson, P.S. (2006). Phenotypic analysis of mice lacking the Tmprss2-encoded protease. Mol Cell Biol, 26(3), 965-75. DOI: 10.1128/MCB.26.3.965-975.2006

- Kuebler, W. M., Parthasarathi, K., Wang, P. M., & Bhattacharya, J. (2000). A novel signaling mechanism between gas and blood compartments of the lung. *The Journal of clinical investigation*, *105*(7), 905-913. doi: 10.1172/JCI8604

- Liu, H., Pan, W., Feng, Y., He, Y., Fu, M., Shi, S., & Sun, C. (2012). Tissue Distribution Study of Bromhexine Hydrochloride Lung-targeting Injectable Microspheres. *LATIN AMERICAN JOURNAL OF PHARMACY*, *31*(10), 1450-1456.

- Lucas, J. M., Heinlein, C., Kim, T., Hernandez, S. A., Malik, M. S., True, L. D., ... & Clegg, N. (2014). The androgen-regulated protease TMPRSS2 activates a proteolytic cascade involving components of the tumor microenvironment and promotes prostate

cancer metastasis. *Cancer discovery*, *4*(11), 1310-1325. DOI: 10.1158/2159-8290.CD-13-1010

- Malerba, M., & Ragnoli, B. (2008). Ambroxol in the 21st century: pharmacological and clinical update. *Expert opinion on drug metabolism & toxicology*, *4*(8), 1119-1129. DOI: 10.1517/17425255.4.8.1119

- McGonagle, D., Sharif, K., O'Regan, A., & Bridgewood, C. (2020). Interleukin-6 use in COVID-19 pneumonia related macrophage activation syndrome. *Autoimmunity reviews*, 102537. DOI: 10.1016/j.autrev.2020.102537
- Miyatake, Y., Makino, S., Kubota, K., Egi, M., & Mizobuchi, S. (2017). Association between Intra-Circuit Activated Clotting Time and Incidence of Bleeding Complications during Continuous Renal Replacement Therapy using Nafamostat Mesilate: a Retrospective Pilot Observational Study. *Kobe Journal of Medical Sciences*, *63*(1), E30.

- Muto, S., Imai, M., & Asano, Y. (1994). Mechanisms of the hyperkalaemia caused by nafamostat mesilate: effects of its two metabolites on Na+ and K+ transport properties in the rabbit cortical collecting duct. *British journal of pharmacology*, *111*(1), 173-178. doi: 10.1111/j.1476-5381.1994.tb14040.x

- Ovcharenko, A. V., & Zhirnov, O. P. (1994). Aprotinin aerosol treatment of influenza and paramyxovirus bronchopneumonia of mice. *Antiviral research*, *23*(2), 107-118. https://doi.org/10.1016/0166-3542(94)90038-8

- Paoloni-Giacobino, A., Chen, H., Peitsch, M. C., Rossier, C., & Antonarakis, S. E. (1997). Cloning of the TMPRSS2 gene, which encodes a novel serine protease with transmembrane, LDLRA, and SRCR domains and maps to 21q22. 3. *Genomics*, *44*(3), 309-320. DOI: 10.1006/geno.1997.4845

- Ramsey, M. L., Nuttall, J., & Hart, P. A. (2019). A phase 1/2 trial to evaluate the pharmacokinetics, safety, and efficacy of NI-03 in patients with chronic pancreatitis:

study protocol for a randomized controlled trial on the assessment of camostat treatment in chronic pancreatitis (TACTIC). *Trials*, *20*(1),501. DOI: 10.1186/s13063-019-3606-y

- Rosseau, S., Selhorst, J., Wiechmann, K., Leissner, K., Maus, U., Mayer, K., ... & Lohmeyer, J. (2000). Monocyte migration through the alveolar epithelial barrier: adhesion molecule mechanisms and impact of chemokines. *The Journal of Immunology*, *164*(1), 427-435. DOI: 10.4049/jimmunol.164.1.427

- Sanders, J. M., Monogue, M. L., Jodlowski, T. Z., & Cutrell, J. B. (2020). Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a review. *Jama*. DOI: 10.1001/jama.2020.6019

- Seifart, C., Clostermann, U., Seifart, U., Müller, B., Vogelmeier, C., von Wichert, P., & Fehrenbach, H. (2005). Cell-specific modulation of surfactant proteins by ambroxol treatment. *Toxicology and applied pharmacology*, *203*(1), 27-35. DOI: 10.1016/j.taap.2004.07.015

- Shen, L. W., Mao, H. J., Wu, Y. L., Tanaka, Y., & Zhang, W. (2017). TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie*, *142*, 1-10. DOI: 10.1016/j.biochi.2017.07.016

- Simoes e Silva, A. C., Silveira, K. D., Ferreira, A. J., & Teixeira, M. M. (2013). ACE2, angiotensin-(1-7) and M as receptor axis in inflammation and fibrosis. *British journal of pharmacology*, *169*(3), 477-492. DOI: 10.1111/bph.12159

- Sonawane, K., Barale, S. S., Dhanavade, M. J., Waghmare, S. R., Nadaf, N. H., Kamble, S. A., ... & Unit, S. B. (2020). Homology modeling and docking studies of TMPRSS2 with experimentally known inhibitors Camostat mesylate, Nafamostat and Bromhexine hydrochloride to control SARS-Coronavirus-2.

- Thienemann, F., Pinto, F., Grobbee, D. E., Boehm, M., Bazargani, N., Ge, J., & Sliwa, K. (2020). World Heart Federation Briefing on Prevention: Coronavirus Disease 2019 (COVID-19) in low-income countries. *World Heart Journal*, *15*(1), 23. http://doi.org/10.5334/gh.778

- Tikellis, C., Johnston, C. I., Forbes, J. M., Burns, W. C., Burrell, L. M., Risvanis, J., & Cooper, M. E. (2003). Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. *Hypertension*, *41*(3), 392-397. DOI: 10.1161/01.HYP.0000060689.38912.CB

- Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G., & Turner, A. J. (2000). A human homolog of angiotensin-converting enzyme cloning and functional expression as a captopril-insensitive carboxypeptidase. *Journal of Biological Chemistry*, *275*(43), 33238-33243. DOI: 10.1074/jbc.M002615200

- Uchiba, M., Okajima, K., Murakami, K., Okabe, H., & Takatsuki, K. (1997). Effect of nafamostat mesilate on pulmonary vascular injury induced by lipopolysaccharide in rats. *American journal of respiratory and critical care medicine*, *155*(2), 711-718. DOI: 10.1164/ajrccm.155.2.9032217

- Vaarala, M. H., Porvari, K. S., Kellokumpu, S., Kyllönen, A. P., & Vihko, P. T. (2001). Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. *The Journal of pathology*, *193*(1), 134-140. DOI: 10.1002/1096-9896(2000)9999:9999<::aid-path743>3.0.co;2-t

- Wan, S., Yi, Q., Fan, S., Lv, J., Zhang, X., Guo, L., ... & Qiang, M. (2020). Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). *MedRxiv*. doi: https://doi.org/10.1101/2020.02.10.20021832

- Westaby, S. (1993). Aprotinin in perspective. *The Annals of thoracic surgery*, *55*(4), 1033-1041. https://doi.org/10.1016/0003-4975(93)90149-C

- Williams, V. R., & Scholey, J. W. (2018). Angiotensin-converting enzyme 2 and renal disease. *Current opinion in nephrology and hypertension*, *27*(1), 35-41. DOI: 10.1097/MNH.0000000000000378

- World Health Organization. (2015). Global status report on road safety; 29(2),142-51.

- World Health Organization. (2020). *Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance, 13 March 2020* (No. WHO/2019-nCoV/clinical/2020.4). World Health Organization.

- World Health Organization. (2020). Coronavirus disease 2019 (COVID-19): situation report, 51.

- Wu, X., Li, S., Zhang, J., Zhang, Y., Han, L., Deng, Q., & Wan, X. (2014). Meta-analysis of high doses of ambroxol treatment for acute lung injury/acute respiratory distress syndrome based on randomized controlled trials. *The Journal of Clinical Pharmacology*, *54*(11), 1199-1206.

- Wu, Z., & McGoogan, J. M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *Jama*. DOI: 10.1001/jama.2020.2648

- Xu, P., Sriramula, S., & Lazartigues, E. (2011). ACE2/ANG-(1–7)/Mas pathway in the brain: the axis of good. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *300*(4), R804-R817. https://doi.org/10.1152/ajpregu.00222.2010

- Yamamoto, M., Kiso, M., Sakai-Tagawa, Y., Iwatsuki-Horimoto, K., Imai, M., Takeda, M., ... & Matsuda, Z. (2020). The anticoagulant nafamostat potently inhibits SARS-CoV-2 infection in vitro: an existing drug with multiple possible therapeutic effects. *bioRxiv*. doi: https://doi.org/10.1101/2020.04.22.054981

- Yamamoto, M., Matsuyama, S., Li, X., Takeda, M., Kawaguchi, Y., Inoue, J. I., & Matsuda, Z. (2016). Identification of nafamostat as a potent inhibitor of Middle East respiratory syndrome coronavirus S protein-mediated membrane fusion using the split-protein-based cell-cell fusion assay. *Antimicrobial agents and chemotherapy*, *60*(11), 6532-6539. DOI: 10.1128/AAC.01043-16

- Yeo, C., Kaushal, S., & Yeo, D. (2020). Enteric involvement of coronaviruses: is faecal–oral transmission of SARS-CoV-2 possible?. *The Lancet Gastroenterology & Hepatology*, *5*(4), 335-337. DOI: 10.1016/S2468-1253(20)30048-0

- Zanasi, A., Mazzolini, M., & Kantar, A. (2017). A reappraisal of the mucoactive activity and clinical efficacy of bromhexine. *Multidisciplinary respiratory medicine*, *12*(1), 7.

- Zheng, Y. Y., Ma, Y. T., Zhang, J. Y., & Xie, X. (2020). COVID-19 and the cardiovascular system. *Nature Reviews Cardiology*, 1-2. DOI: 10.1038/s41569-020-0360-5

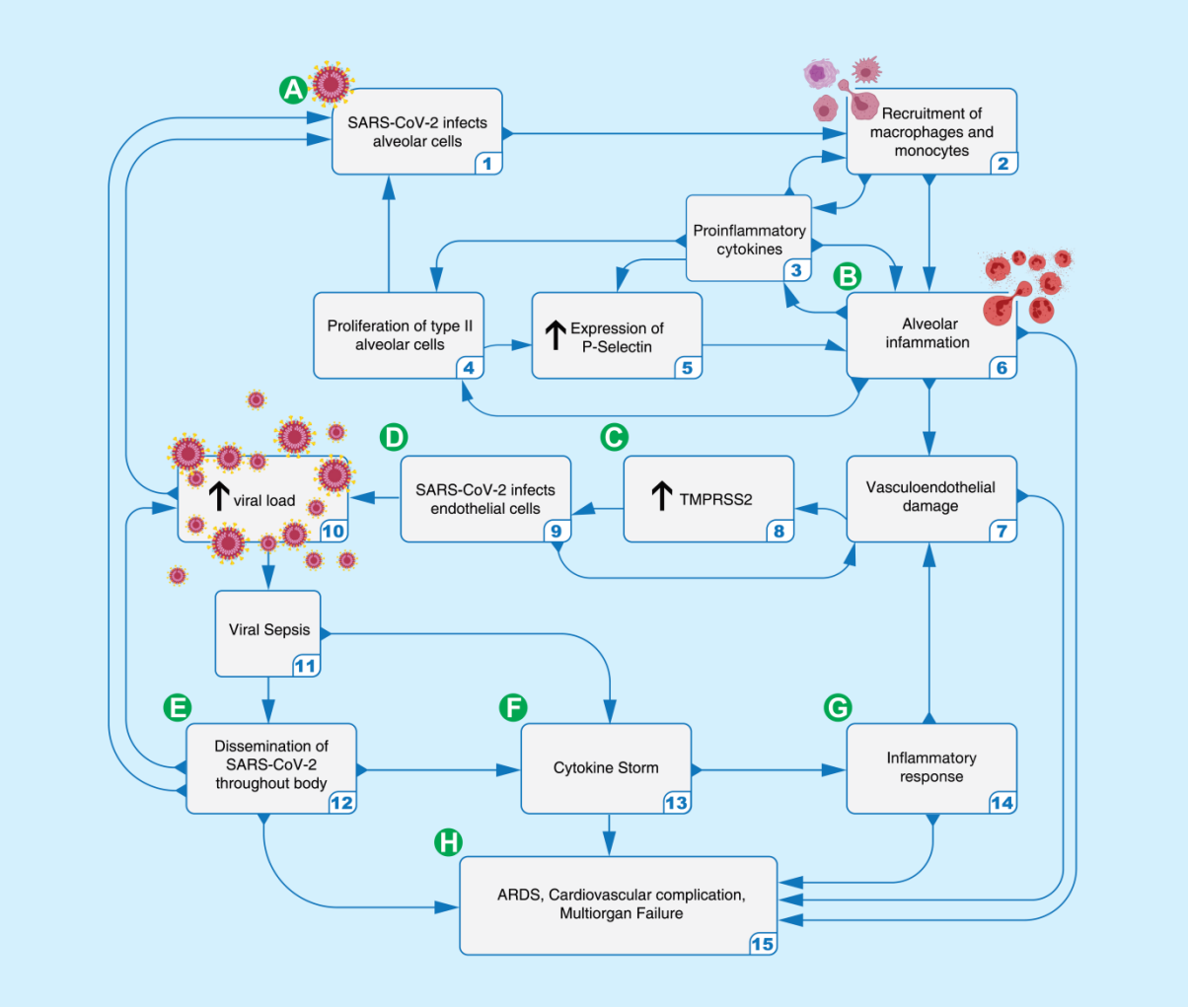

Figure 1: 1. SARS-CoV-2 infects alveolar cells. **2.** Type II alveolar cells chemotactically attract macrophage and monocytes. **3.** Macrophages and monocytes release chemoattractants and proinflammatory cytokines. **4.** Chemoattractants and proinflammatory cytokines stimulate proliferation of type II alveolar cells. **5.** Type II alveolar cells stimulate expression of P-Selectin in perialveolar capillary network. **6.** Chemoattraction of immune cells and subsequent alveolar inflammation stimulate proliferation of type II alveolar cells, constituting a vicious cycle. **7.** Inflammation causes vasculoendothelial damage. **8.** Expression of TMPRSS2 increases in endothelium of damaged vessels. **9.** SARS-CoV-2 infects inflamed endothelial cells to a higher extent. **10.** Another vicious cycle links vasculoendothelial damage, increased expression of TMPRSS2 and increased endothelial infection together and causes high viral load. **11.** Viruses find their way to circulation and cause viral sepsis. **12.** Viral sepsis accelerates dissemination of SARS-CoV-2 throughout body. **13.** Over-reaction of immune system to high viral burden leads to cytokine Storm. **14.** Cytokine storm causes systemic inflammatory response. **15.** Systemic inflammatory response is complicated by ARDS, cardiovascular complication, and multiorgan Failure. **A.** TMPRSS2 inhibitors,

Bromhexine, Aprotinin, Camostat and Nafamostat hinder implantation of SARS-CoV-2 in alveolar cells. **B.** Nafamostat and Ambroxol, reduce alveolar inflammation and alleviate ARDS. **C.** TMPRSS2 inhibitors, inactivate expressed TMPRSS2. **D.** TMPRSS2 inhibitors hinder implantation of SARS-CoV-2 in endothelial cells, reduce vasculoendothelial damage and break the vicious cycle of vasculoendothelial damage, increased expression of TMPRSS2, and endothelial infection. **E.** TMPRSS2 inhibitors hinder dissemination and secondary replication of SARS-CoV-2 throughout body. **F.** Nafamostat and Ambroxol down regulate expression of IFNγ, TNF-alpha, IL-1β, IL-6, and IL-8. **G.** Nafamostat and Amroxol alleviate systemic inflammatory response. **H.** TMPRSS2 inhibitors block all the steps which contribute to ARDS, Cardiovascular complications and multiorgan failure.