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LETTERS

Nitazoxanide/Camostat combination for COVID-19: An unexplored potential therapy

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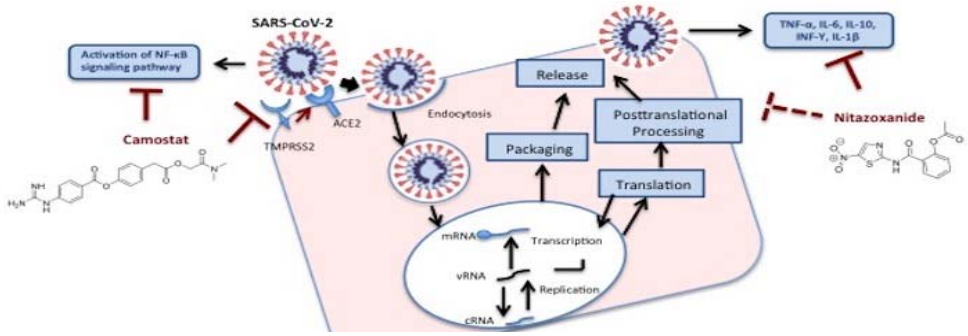
ABSTRACT



Novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lies behind the ongoing outbreak of coronavirus disease 2019 (COVID-19) and its ripple effects has brought major challenges to worldwide health systems. Urgent strategies are required to manage this pandemic and repurposing of existing drugs offer an option forward. A combination drug therapy for COVID-19 aims to prevent both the virus entry/spread as well as quelling the immune system havocs that the virus wreaks in the human body. This article analyzes Nitazoxanide/Camostat combination for their potential activity against SARS-CoV-2. Nitazoxanide, FDA approved drug potentiates host antiviral response, thereby reducing viral replication, titer and ensuing immune dysregulation. Camostat, a potent serine protease inhibitor blocks viral cell entry along with the potential to decrease COVID-19-associated hypercoagulability. Both the drugs do not inhibit CYP 450 enzymes and could be co-administered. Based on the combined pathophysiological and pharmacological potential, the drugs combination can prospectively be recommended for early evaluation with possible clinical trials of this combination.

Keywords: COVID-19; Nitazoxanide; Camostat; Hypercoagulability; SARS-CoV-2.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the ongoing coronavirus disease 2019 (COVID-19) pandemic, is continuing its spread across the world; with more than eleven million confirmed cases in 188 countries.[1] SARS-Cov-2, novel β-coronavirus, is an enveloped virus with a positive-polarity, single-stranded RNA genome. It shares strong similarities with two previous highly pathogenic human β-coronaviruses, SARS-CoV and MERS-CoV. SARS-CoV-2 shares approximately 79% and 50% sequence identity with SARS-CoV and MERS-CoV, respectively,[2] similar cell entry mechanisms,[3] and the propensity to induce hyper inflammation in severe cases.[4] Notably, SARS-CoV-2 has lower pathogenicity than SARS-CoV but higher transmissibility from human to human.[5] Nearly 500,000 people have lost their lives. In addition to developing new treatment options, numerous existing antiviral agents, immunotherapies, and vaccines are under investigation against SARS-CoV-2.[6] Unfortunately, standard treatment against COVID-19 is still lacking. Hence, it remains a major challenge to decide what potential therapeutic regimens to be used for the prevention and treatment of COVID-19 patients.

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The first and foremost strategy for the treatment of COVID-19 is the repurposing of existing drugs that have already been shown to be safe in humans. Drug repurposing, is defined as identifying alternative uses for approved or investigational drugs outside their defined indication, could be a possible way to overcome the time limitation of research and development needed to design a therapeutic drug to combat the pathogen.[7] The use of these drugs fast tracks a treatment plan for COVID-19, as they have known toxicity and safety profiles too. In the current scenario, it seems increasingly unlikely that a single drug would be able to cure COVID-19. It can be through combination of drugs, as in the past we have beaten TB and HIV through combination of antibiotics or antiretroviral respectively, we can beat COVID-19 as well.

Combination drugs attack COVID-19 through multiple ways and improves the treatment efficacy. For example, Antivirals can stop the virus from multiplying in humans but alone they are moderately able to improve the patient's health. An ideal management of COVID includes an antiviral, which can stop the virus from multiplying in humans and other drug, which can take care of immunological issues arising with infection without any drug-drug interaction. A recent study found that a combination of three drugs — interferon beta-1b, lopinavir-ritonavir, and ribavirin — plus standard care is successful in treating mild-to-moderate cases of COVID-19.[8] Globally there are many clinical trials underway to evaluate the efficacy of a number of repurposed drugs in combination, against SARS-CoV-2.[9-11] This article illustrates and analyzes the rationale for the FDA-approved antidiarrheal drug; nitazoxanide (figure 1) to be tested in combination with serine protease inhibitor camostat (figure 1) for their potential activity against SARS-CoV-2.

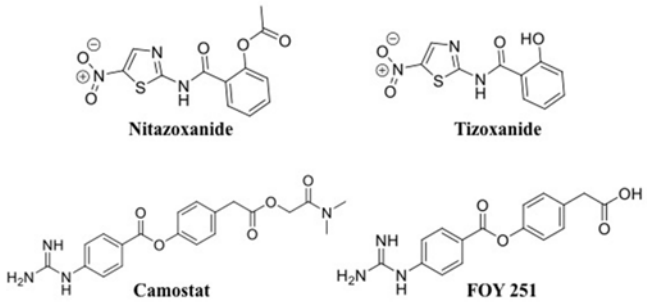

Figure 1. Chemical structure of Nitazoxanide, Camostat and their metabolites

Host Cell Infection and Disease Pathogenesis

SARS-CoV-2 is transmitted primarily via respiratory droplets and contact routes. The infection starts with virus binding to a host cell through its target receptor. The virus principally targets airway epithelial cells, alveolar epithelial cells, vascular endothelial cells and macrophages in the lung, all of which express the angiotensin-converting enzyme 2 (ACE2) host target receptor. SARS-CoV-2 binds to two host surface-expressed proteins, ACE2 and the serine protease, TMPRSS2, through its Spike (S) protein. The viral S protein is cleaved into two functional subunits, S1 that interacts with ACE2, and S2 that is further cleaved and activated by TMPRSS2. Together, these actions result in viral-host membrane fusion.[3] These high-affinity interactions are essential in viral entry and are therefore prime targets in the treatment of COVID-19.

SARS-CoV-2 entry into the alveolar epithelium triggers innate immune response. Recognition of the virus by innate immune receptors, such as RNA sensors TLR7/8 and RIG-I/MDA-5, and the inflammasome sensor, leads to activation of NF-kB and IRF3/7 and the subsequent production of proinflammatory cytokines (eg. IL-1B and IL-6) and type 1 IFNs respectively.[12] Induction of the type I IFN response is essential in limiting the propagation of the virus within the host during the early phases of the disease, but downregulation of the host IFN response, either directly by the virus or by other indirect means, can cause an unbalanced production of pro-inflammatory cytokines and infiltration of inflammatory cells leading to a more severe form of COVID-19 (figure 2).[13,14]

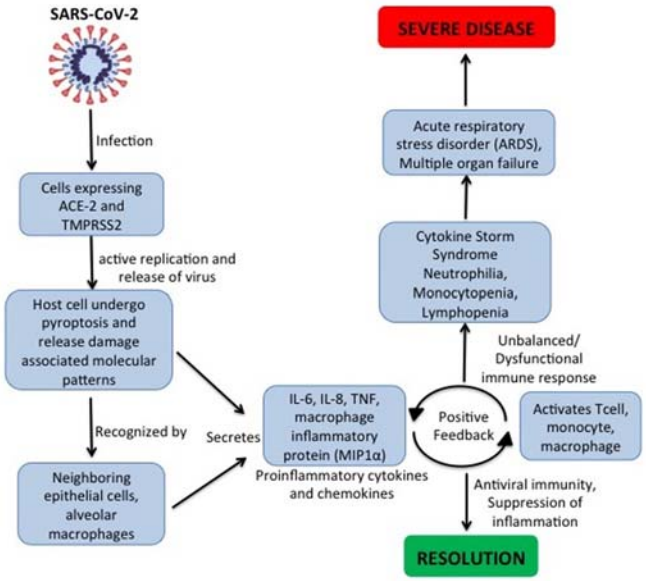

Figure 2. Schematic representation of chronology of events during SARS-CoV-2 infection

Cytokines released by infected cells modulate the adaptive immune response by recruiting and activating immune cells such as macrophages, B cells and T cells to orchestrate the elimination of the virus. However an unbalanced and defective immune response lead to further accumulation of immune cells in the lungs, causing overproduction of pro-inflammatory cytokines, which eventually damages the lung infrastructure. Pulmonary recruitment of immune cells from the blood and the infiltration of lymphocytes into the airways may explain the lymphopenia and increased neutrophil–lymphocyte ratio seen in around 80% of patients with SARS-CoV-2 infection.[15,16] The resulting cytokine storm circulates to other organs, leading to multi-organ damage (figure 2).

NITAZOXANIDE TARGET HOST ANTIVIRAL RESPONSE AGAINST COVID-19

Nitazoxanide or 2-(acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide (Fig. 1) was first synthesized in the early 1970s on the scaffold of niclosamide in replacing one benzene ring, a 6-membered ring heterocycle, by a nitrothiazole, a 5-membered ring heterocycle (figure 1).[17] It was originally developed and commercialized as an antiprotozoal agent, which was later identified as a first-in-class broad-spectrum antiviral drug and has been repurposed for the treatment of influenza. It has also been widely commercialized in Latin America and in India where it is indicated for treating a broad spectrum of intestinal parasitic infections. Nitazoxanide is an orally bioavailable and safe thiazolide, which rapidly gets hydrolyzed by plasma esterases to form its active circulating metabolite tizoxanide (figure 1).[18] The drug is eliminated in urine and bile as tizoxanide and tizoxanide glucuronide. The half-life of tizoxanide in plasma is only approximately 1.3h. More than 99.9% of circulating tizoxanide is bound to plasma proteins.[19]

Nitazoxanide and its metabolite both exhibit *in vitro* inhibitory activity against rotaviruses, hepatitis B and C viruses, noroviruses, influenza viruses, MERS, and SARS,[20] is the primary reason of its selection for the combination. The mechanism of action of Nitazoxanide for SARS-CoV-2 is currently unknown. However, for influenza it has been reported to involve interference with N-glycosylation of hemagglutinin.[21] Since the SARS-CoV-2 spike protein is also heavily glycosylated with similar cellular targets in the upper respiratory tract, a similar mechanism of action may be expected (figure 3).[22]

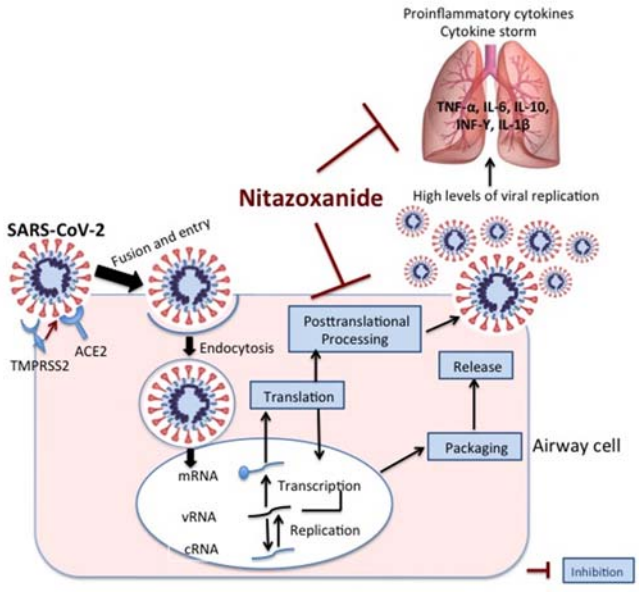

Figure 3. Schematic representation of plausible mechanism of Nitazoxanide in the pathogenesis of COVID-19

Other reason for selecting Nitazoxanide against SARS-CoV-2 could be derived from its impact on immune system in potentiating the production of type 1 interferons[23] and bronchodilation of the airways through inhibition of TMEM16A ion channels.[24] In addition, It inhibits the production of pro-inflammatory cytokines TNFα, IL-2, IL-4, IL-5, IL-6, IL-8 and IL-10 in peripheral blood mononuclear cells (figure 3).[19] Nitazoxanide has been tested in clinical setting for the treatment of acute uncomplicated influenza, where the patients received either 600 mg or 300 mg of Nitazoxanide or placebo orally twice daily for five days and were followed for 28 days. Patients who received Nitazoxanide 600 mg twice daily experienced less times for alleviation of symptoms as compared with patients who received 300 mg Nitazoxanide twice daily, which, was even shorter than placebo. Nitazoxanide is relatively safe in humans and studies showed tolerability of single oral doses up to 4 g with minimal gastrointestinal side effects.[25] Plasma concentrations of tizoxanide have demonstrated dose proportionality, but administration in the fed state increases the plasma exposure.[26] *In vitro* metabolism studies have demonstrated that tizoxanide has no significant inhibitory effect on cytochrome P450 enzymes, thus it is expected that no significant interaction would occur when nitazoxanide is co-administered with drugs that either are metabolized by or inhibit cytochrome P450 enzymes.[19]

CAMOSTAT, A SERINE PROTEASE INHIBITOR, BLOCKS CELL ENTRY

Camostat Mesylate (figure 1) is the mesylate salt form of camostat an orally bioavailable, synthetic serine protease inhibitor, with anti-inflammatory, antifibrotic, and potential antiviral activities.[27] Upon oral administration, camostat and its metabolite 4-(4-guanidinobenzoyloxyl)phenyl acetic acid (FOY 251) (figure 1) inhibit the activities of a variety of proteases, including trypsin, kallikrein, thrombin and plasmin, and C1r- and C1 esterases.[28] Camostat is approved in Japan for the treatment of chronic pancreatitis and postoperative reflux esophagitis.[29] Camostat inhibits the activity of transmembrane protease, serine 2 (TMPRSS2), a host cell serine protease that mediates viral cell entry for coronavirus, thereby inhibiting viral infection and replication (figure 4). It has shown broad-spectrum activity against enveloped RNA viruses such as CoVs and paramyxoviruses. It is reported to inhibit SARS and MERS in *ex vivo* studies and improves the survival of mice infected with SARS.[30,31] Hoffmann et al. determined that the SARS-CoV-2 requires TMPRSS2, furthermore, using a sample of SARS-CoV-2 virus isolated from a patient, they found that Camostat blocks the entry of the virus into the lung cells and reduces the number of genomic equivalents of SARS-CoV-2, a marker of infection, in Calu-3 cells.[3] Another *in vivo* study showed that Camostat inhibits the function of the sodium channel in human respiratory epithelial cells (IC50 = 50nM) and improves mucociliary clearance in sheep.[32] In mice, camostat mesilate dosed at concentrations similar to the clinically achievable concentration in humans reduced mortality following SARS-CoV infection from 100% to 30-35%.[33] Researchers at Cancer Research UK and its partners have initiated a clinical trial to study camostat, as a potential therapy against Covid-19.[34]

Camostat mesilate, a potent serine protease inhibitor, inhibits trypsin and various inflammatory proteases including plasmin, kallikrein and thrombin and suppresses pancreatitis-induced pain in rats following oral administration. Administration of camostat (1 mg/ kg) inhibits the production of tumor necrosis factor-α (TNF-α) and monocyte chemo attractant protein-1 by monocytes and the proliferation of pancreatic star cells in a rat model of pancreatic fibrosis.[35] Camostat mesilate after oral administration acts on kinin formation, fibrinolytic effects, coagulopathy and complementary system to immediately inhibit enzyme activities and their abnormal increases.[36] Camostat mesilate and its metabolites did not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, indicating no significant drug interaction.[37] Camostat is licensed in Japan and South Korea to treat chronic pancreatitis, so it can be expected to enable rapid manufacturing, if successful in treating Covid-19.

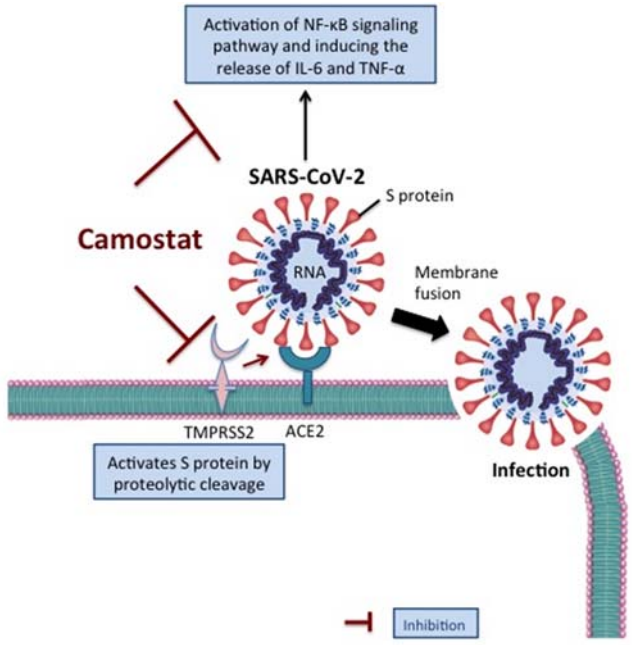

Figure 4. Schematic representation of plausible mechanism of Camostat in the pathogenesis of COVID-19

CONCLUSION

Controlling the inflammatory response could be as important as targeting the virus in the treatment of COVID-19. Therapies inhibiting viral infection and regulation of dysfunctional immune responses may synergize to block pathologies at multiple steps. In conclusion, the rationale for this combination therapy stems from Nitazoxanide's broad-spectrum antiviral activity, its role in up regulation of innate immune response to prevent ongoing viral replication in infected cells and its safety profile. Camostat acts as inhibitor of the host cell serine protease TMPRSS2, which is needed to prime the viral S protein for cell entry; thus blocking viral entry in lung cells. Also, Camostat's prompt action on fibrinolytic, coagulation and complementary systems to immediately inhibit enzyme activities could be beneficial in reducing COVID-19-associated hypercoagulability in severe cases. Low cost and easy availability of both the drugs is the biggest advantage of this combination. The authors hope that exploration of this potential combination would help in paving the way in finding an effective treatment of COVID-19.

Conflict of interest: The authors declare that there are no conflicts of interest.

REFERENCES

1. Novel Coronavirus (2019-nCoV) Situation. Report-170. World Health Organisation; [Accessed 8th July 2020]
2. R. Lu, X. Zhao, J. Li et al. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* **2020**, 395, 565-574.
3. M. Hoffmann, H. Kleine-Weber, S. Schroeder, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **2020**, 181(2), 271-280.
4. C. Huang, Y. Wang, X. Li et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **2020**, 395, 497-506.
5. Q. Li, X. Guan, P. Wu, X. Wang, L, Zhou, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med.* **2020**, 382(13), 1199-1207.
6. S. G. Viveiros Rosa, W. C. Santos. Clinical trials on drug repositioning for COVID-19 treatment. *Pan American J. Public Health.* **2020**, 44, 40.
7. V. Chandel, S. Raj, B. Rathi, D. Kumar. In silico identification of potent FDA approved drugs against coronavirus COVID-19 main protease: A drug repurposing approach. *Chem. Biol. Lett.*. **2020**, 7(3), 166-175.
8. I.F. Hung, K. Lung, E. Y. Tso, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet.* **2020**, 395(10238), 1695-1704.
9. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). **2020** Feb 5, Identifier NCT04252274, Efficacy and Safety of Darunavir and Cobicistat for Treatment of COVID-19 (DC-COVID-19).
10. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). March 11, **2020**, Identifier NCT04303299, Various Combination of Protease Inhibitors, Oseltamivir, Favipiravir, and Hydroxychloroquine for Treatment of COVID-19: A Randomized Control Trial (THDMS-COVID-19).
11. ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). March 2, **2020**, Identifier NCT04291729, Evaluation of Ganovo (Danoprevir) Combined With Ritonavir in the Treatment of SARS-CoV-2 Infection.
12. K. Bunte, T. Beikler. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. *Int. J. Mol. Sci.* **2019**, 20, 3394.
13. C.Y. Cheung, L.L.Poon, I.H. Ng, et al. et al. Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. *J. Virol.* **2005**, 79, 7819-7826.
14. W. M. Schneider, M. D. Chevillotte, C. M. Rice, et al. 2014. Interferon-stimulated genes: a complex web of host defenses. *Annu. Rev. Immunol.* **2014**, 32, 513-545.
15. C. Qin, L. Zhou, Z. Hu, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin. Infect. Dis.***2020**, 12, 248.
16. B. S. Chhikara, B. Rathi, J. Singh, Poonam. Corona virus SARS-CoV-2 diasesa COVID-19: Infection, Prevention and clinical advances of the prospective chemical drug therapeutics. *Chem. Biol. Lett*. **2020**, 7(1), 63-72.
17. J. F. Rossignol, R, Cavier. Synthesis and antiparasitic activity of 2-benzamido nitrothiazoles. *Chem. Abstr*. **1975**, 83, 28216.

18. J. Broekhuysen, A. Stockis, R. L. Lins, et al. Nitazoxanide: pharmacokinetics and metabolism in man. Int. *J. Clin.. Pharmacol*. **2000**, 38, 387–394.
19. Romark Laboratories. L.C. Alinia® (nitazoxanide) prescribing information
20. J. F. Rossignol. Nitazoxanide: A first-in-class broad-spectrum antiviral agent. *Antivir. Res.* **2014**, 110, 94-103.
21. J. F. Rossignol, S, La Frazia, L, Chiappa, et al. Thiazolides, a new class of anti-influenza molecules targeting viral hemagglutinin at post-translational level. *J. Biol. Chem.* **2009**, 284, 29798–29808.
22. R. K. R. Rajoli, H. Pertinez, U. Arshad, et al. Dose prediction for repurposing nitazoxanide in SARS-CoV-2 treatment or chemoprophylaxis. *medRxiv* **2020**, 05.01.2008713.
23. M. Clerici, D. Trabattoni, M. Pacei, et al. The anti-infective Nitazoxanide shows strong immumodulating effects. *J. Immunol.* **2011**, 186(1 Supplement), 155, 21.
24. K. Minar, K. Labitzke, B. Liu, et al. Drug Repurposing: The Anthelmintics Niclosamide and Nitazoxanide Are Potent TMEM16A Antagonists That Fully Bronchodilate Airways. *Front. Pharmacol* **2019,** 10, 51-51.
25. J. Haffizulla, A. Hartman, M. Hoppers, et al. Effect of nitazoxanide in adults and adolescents with acute uncomplicated influenza: a double-blind, randomised, placebo-controlled, phase 2b/3 trial. *Lancet Infect. Dis.* **2014,** 14(7), 609-18.
26. A. Stockis, A. M.. Allemon, S. De Bruyn, et al. Nitazoxanide pharmacokinetics and tolerability in man using single ascending oral doses. *Int. J. Clin. Pharmacol. Ther.* **2002,** 40(5), 213-220
27. B.D. Savi, D. L. Hughes, L. Kvaerno. Quest for a COVID-19 Cure by Repurposing Small-Molecule Drugs: Mechanism of Action, Clinical Development, Synthesis at Scale, and Outlook for Supply. *Org. Process Res. Dev.* **2020,** *24* (6), 940-976.
28. Y, Tamura, M, Hirado, K. Okamura, et al. Synthetic inhibitors of trypsin, plasmin, kallikrein, thrombin, C1r-, and C1 esterase. *Biochim Biophys Acta* **1977**, 484, 417–422.
29. S. Ota, Y. Hara, S. Kanoh, et al. Acute eosinophilic pneumonia caused by camostat mesilate: The first case report. *Respir. Med. Case Rep*. **2016**, 16, 21-23
30. Y. Zhou, P. Vedantham, K. Lu, J. Agudelo, R. Jr. Carrion, et al. Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res*, **2015,** 116, 76-84.
31. M, Yamamoto, S. Matsuyama, X, Li, M. Takeda, et al. 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. *Antimicrob Agents Chemother*. **2016,** 60, 6532-6539.
32. K. Coote, H. C. Atherton-Watson, R. Sugar, et al. Camostat attenuates airway epithelial sodium channel function in vivo through the inhibition of a channel-activating pro- tease. *J. Pharmacol. Exp. Ther*. **2009**, 329(2), 764-774.
33. Y. Zhou, P. Vedantham, K. Lu, et al. Protease inhibitors targeting coronavirus and filovirus entry. *Antiviral Res.* **2015**, 116, 76–84.
34. Clinicaltrialsregister.eu [Internet]. May 29, **2020**, EudraCT Number, 2020-002110-41, SPIKE-1 TRIAL: A Randomised Phase II/III trial in a community setting, assessing use of camostat in reducing the clinical progression of COVID-19 by blocking SARS-CoV-2 Spike protein-initiated membrane fusion.
35. S. Bittmann. The role of Angiotensin-2 receptor in transmission process. *J Regen Biol Med.* **2020**, 2(1), 1-2.
36. B. Stefan, A. Elena Moschüring, W. Anne, et al. The role of TMPRSS2-Inhibitor Camostat in the pathogenesis of COVID-19 in lung cells. *Biomed J. Sci. & Tech. Res.* **2020,** 27(3).
37. N. Matsunaga, et al. The effect of camostat mesilate on CYP 450 isozymes. Internal data of Ono Pharmaceutical Co., Ltd. Available from: https://www.ono.co.jp/eng/news/pdf/sm_cn200605_3.pdf