STUDY PROJECT
Versione 2- 07.01.2021

A Randomized Clinical Trial of Nafamostat:

A Potent Transmembrane Protease Serine 2 (TMPRSS2) Inhibitor

for the Treatment of Covid-19

Sponsor: Department of Medicine-DIMED, University of Padua

PI: Gian Paolo Rossi,
Department of Medicine-DIMED, University of Padua

Investigators: Viola Sanga,[1,2] Teresa M. Seccia,[1] Yusuke Kobayashi,[3] Roberto Vettor,[1] Matthias Barton,[4,5,] Dario Gregori[6]

[1]Department of Medicine-DIMED, [2] International PhD Program in Arterial Hypertension and Vascular Biology (ARHYVAB) University of Padua, [3] Center for Novel and Exploratory Clinical Trials (Y-NEXT), Yokohama City University, Yokohama, Japan

3rd Department of Medical Science and Cardiorenal Medicine, Yokohama City University Graduate School of Medicine, Yokohama, Japan); [4] [5]University of Zürich and [6]Andreas Grüntzig Foundation, Zürich, Switzerland;

Unit of Biostatistics, Epidemiology and Public Health, Department of Cardiac, Thoracic, Vascular Sciences, and Public Health, University of Padua, Padova, Italy,

Key words: Nafamostat, proteases, TMPRSS2, ACE-2, covid-19

Correspondence to:
Prof. Gian Paolo Rossi, MD. FACC, FAHA
DIMED– Hypertension Unit
University Hospital
Via Giustiniani, 2
35126 Padova, Italy
Phone: 39-049-821-2279 or 7821
Fax: 39-049-821-7873
E-mail: gianpaolo.rossi@unipd.it

Abstract

Purpose: SARS-Cov-2 enters the lung cells by binding to ACE-2 and activating the protease TMPRSS2, which, therefore, can be a target for antiviral treatment. Accordingly, TMPRSS2 inhibitors prevent SARS-CoV cell entry in vitro. The most potent such inhibitors, nafamostat is being used as anticoagulant and is approved for the treatment of cystic fibrosis as its mucolytic action can lowering airways infections. We hypothesize that nafamostat could be extremely useful in COVID-19 lung involvement, which entails activation of the coagulation cascade, pulmonary embolism, and bacterial superinfections. Therefore, we herein report on the design of a prospective trial that will test the hypothesis that nafamostat can lower lung function deterioration and need for intensive care admission in COVID-19 patients.

Design: The study 1 will be at planned doses and times in COVID-19 will consist in a group-sequential double-blind randomized placebo-controlled clinical study in adult hospitalized COVID-19 patients, aimed at testing the clinical efficacy of nafamostat mesylate (administered intravenously as continuous infusion for 7 days) on top of best standard of care.

Primary outcome measures: the time-to-clinical improvement, defined as the time from randomization to a 2-point improvement from randomization on a 7-category ordinal scale.

Conclusion: To our knowledge, no prospective randomized clinical trial has involved inhibitors of TMPRSS2 in COVID-19 to date. Hence, we expect to establish if a TMPRSS2 inhibition strategy with nafamostat is valuable to decrease the hospital stay and lower the rate of COVID-19 patients who need to be admitted to the intensive care units.

Introduction

In June 2016, by using the split-protein-based cell-cell fusion assay, Yamamoto et al. discovered that nafamostat, a potent inhibitor of several proteases, including the transmembrane protease serine 2 (TMPRSS2), effectively prevented the Middle-East Respiratory Syndrome Corona virus (MERS-Cov) infection in vitro.[1] TMPRSS2 is used to enter the lung cells by the MERS-CoV and also by the SARS-Cov, the Corona virus that caused the SARS outbreak and killed 774 patients in 2003.

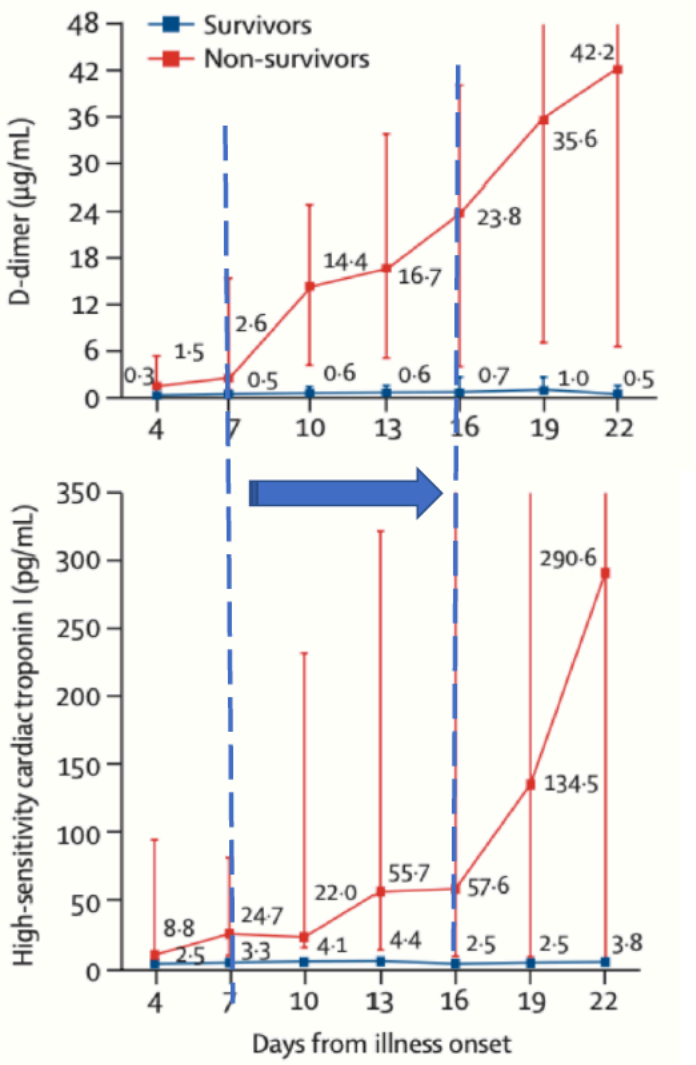
On January 20th 2020 two Chinese groups independently reported the full sequence of the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2), which caused the recent outbreaks of Coronavirus disease 2019 (Covid-19) in Wuhan that rapidly reached pandemic proportions. By phylogenetic analyses the investigators noticed remarkable similarities between the SARS-Cov and the SARS-Cov-2 virus, particularly in terms of their spike (S) protein, an envelope protein that unlike the MERS-Cov is used by both viruses to bind to the angiotensin-converting-enzyme(ACE)-2.[2,3]

The fact that ACE-2 is highly expressed in the lung, particularly in the type 2 alveolar epithelial cells, and in the vascular endothelium,[4] accounted for the peculiar predilection of these viruses for the lung and for causing the deadly multiorgan failure. Electron microscopy-based ultrastructural studies thereafter showed that SARS-Cov-2 binds to ACE-2 with a higher affinity than SARS-Cov,[5] which may account for its higher contagiousness.

In the March issue of Cell Hoffman et al. showed that upon binding to ACE-2, SARS-Cov-2 enters the lung cells by activating TMPRSS2. The ensuing recruitment of the cell molecular machinery allows a rapid replication of the virus and spreading of the infection to the surrounding cells, a phenomenon already well documented for the SARS-Cov.[6] The associated tissue damage and inflammation can release tissue factor and thus activate factor VII of the

extrinsic pathway of the coagulation cascade, with ensuing disseminated intravascular coagulation in the lung and other organs. Importantly, Hoffman et al. also showed that hyperimmune sera of patients who recovered from SARS, and camostat, a TMPRSS2 inhibitor clinically approved in Japan, effectively blunted SARS-Cov-2 entry into the cell.

As mentioned above, nafamostat, another TMPRSS2 inhibitor more potent than camostat, which is approved in Japan for a number of clinical indications, including anticoagulation and chronic pancreatitis, was also found to prevent SARS-CoV cell entry.[1] Noteworthy, nafamostat mesylate is now a generic drug used with a long-standing Japanese experience, which has shown that it is extremely well tolerated, even in patients on chronic hemodialysis (Yusuke Kobayashi, personal communication). Furthermore, at variance with camostat,[7] which can cause eosinophilic pneumonia,[8] and has anti-fibrinolytic actions that could be detrimental in COVID19 patients, nafamostat mesylate has anticoagulant properties[9]. The latter can be extremely useful in COVID-19-infected patients inasmuch as those who did not survive the infection exhibited clear-cut signs of an activation of the coagulation cascade,[10] pulmonary embolism,[11] and myocardial damage evidenced by an increase of D-dimer that preceded that of troponin I.[12] A closer look at the study by Zhou et al., published in The Lancet on March 12th 2020, clearly revealed that besides age, the neutrophil/lymphocyte count, and the SOFA score, the elevation of D-dimer and Troponin I levels clearly identified the non-survivor COVID-19 patients.[12] The following plots provided unambiguous evidence that The D-dimer elevation, a proxy for DIC, anticipated by 6-9 days the myocardial damage evidenced by troponin I.



The temporal changes of plasma concentrations of D-Dimer levels and high sensitivity troponin I. Please note the 7-8 days delay between the onset of DIC and of signs of myocardial damage (data from Zhou et al. [12]).

The European Medicines Agency (EMA) has granted nafamostat mesylate the status of orphan drug for the treatment of children with cystic fibrosis, a conditions featuring increased mucus viscosity caused by enhanced activity of the epithelial sodium (Na^+) channel (eNaC), thus facilitating recurrent pulmonary infections and leading to progressive deterioration of lung function. By inhibiting the eNaC activity in the bronchial epithelium, decreasing sodium secretion in the mucus, and thus mucus viscosity, nafamostat mesylate was judged to be useful

in cystic fibrosis.[7] As in COVID-19 bacterial superinfection has been reported to contribute to worsen the clinical conditions and to predict a fatal course,[12] this ancillary action of nafamostat mesylate can be useful in infected patients.

Hence, the bulk of available data collectively indicate that, besides its unambiguous antiviral properties, nafamostat mesylate has multiple favorable actions, including anticoagulant and mucolytic properties, that can blunt the progression of COVID-19 toward acute respiratory distress syndrome (ARDS) in infected patients.

Considering that currently there are no effective treatments for COVID-19, we herein set out to test the hypothesis that nafamostat mesylate lowers the progression of lung function deterioration and can shorten the need for hospitalization in COVID-19-infected patients in a prospective double-blind randomized clinical trials.

Methods

Study design and purposes

The study will consist in a group-sequential double-blind randomized placebo-controlled group-sequential parallel-arms trial, on top of best standard of care. The trial has been designed to comply with all requirement of the CONSORT checklist for high-quality randomized clinical trial (Supplemental Material)[13] and has been registered at clinicaltrial.gov (Identifier: NCT04352400).

The primary endpoint is to assess the clinical efficacy of nafamostat mesylate in patients with severe COVID-19 patients defined as at least a 2-point improvement in a clinically validated 7-category ordinal scale[14] (Table 1).

Secondary Objectives are to evaluate the effect on the clinical safety and biomarker changes (e.g. IL-6, Ang II) in patient with severe COVID-19 treated with nafamostat mesylate.

The study will comprise 2 treatment groups as follows: Group A (active): nafamostat mesylate (0.10 mg/kg/h i.v.); Group B (placebo control): sterile, 5% dextrose i.v..

Eligible patients with a confirmed diagnosis of COVID-19, as defined below, will be randomly allocated (1:1), to Group A or B to receive the treatment or placebo, at baseline (day 1). Randomization will be done with an algorithm tailored to the study design. Investigators and patients will be blinded to the treatment administered.

Patients

After screening at day - 1, the patients will be treated with nafamostat mesylate or placebo on day 1 and will be dosed thereafter until day 7 as specified below. Follow up visits will be done at a daily basis for the first seven days and then on day 10, 14 and 28 to asses efficacy and safety. The study assessment will be performed according to Tables 2 and 3.

With a half-life of 8 minutes[15] nafamostat mesylate is rapidly eliminated from the blood. Hence, we have planned to administer it intravenously as a continuous infusion over 24 hours for seven calendar days at a dose of 0.10 mg/Kg/h dissolved in 1000 ml 5% dextrose. The dose can be decreased (down to 0.05 mg/Kg/h) or increased (up to 0.20 mg/kg) according to symptoms, blood pressure values, and clinical response. Blood pressure will be continuously monitored noninvasively with automated devices throughout the infusion. Serum potassium levels will be measured after the first 6 hours of infusion and daily during the 7 days of the drug administration in both treatment groups.

The anonymized data will be entered and stored securely in an ad hoc created web-based collection data form and stored securely in a server protected with firewalls and passwords as done for other multicenter studies run by the PI.[16–18]

Definitions

The diagnosis of COVDI19 will be based on positive（SARS-CoV-2 nucleic acid –qPCR). Responder to treatment is defined as ≥ 2 improvement in a seven-category ordinal scale at day 7, 10, 14 and 28.[14]

Inclusion criteria

We will include in the study patients of both sexes if the following criteria apply:

- Hospitalized, COVID-19 positive, between 18 and 85 years of age;
- Signed Inform Consent Form;
- Body temperature > 37.3 ℃;
- Oxygenation criterion (any of the following): i) Oxygen saturation ≤94% on Room Air; ii) PaO2/FiO2 ratio ≤300 mmHg but > 100 mmHg, if patient on supplemental oxygen; iii) SpO2/FiO2<200 if no arterial blood gas available;
- Respiratory rate (RR) ≥ 25 beats/min.

Exclusion criteria

- Pregnant or lactating females;
- Unwillingness or inability to complete the study.
- Rapidly deteriorating clinical condition or low likelihood to complete the study according to the investigator;
- eGFR < 30 ml/min/m2 assessed with CKD EPI formula;
- Current or chronic history of liver disease (Child Pugh score ≥ 10), or known hepatic or biliary abnormalities;
- Participation in a clinical trial with an investigational product within the following time period prior to the first dosing day in the current study: 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer);

- Patients requiring high doses of loop diuretics (i.e. > 240 mg furosemide daily) with significant intravascular volume depletion, as assessed clinically;
- History of allergy;
- History of sensitivity to heparin or heparin-induced thrombocytopenia;
- Unstable hemodynamics in the preceding 4 hours (MAP < 65 mmHg, or SAP < 90 mmHg, DAP < 60 mmHg, and/or vasoactive agents required);
- Hemoglobin < 7 g/dL at time of drug infusion. Transfusion is allowed to increase hemoglobin levels before entry into the study;
- Malignancy or any other condition for which estimated 6-month mortality >50%;
- Arterial blood pH less than 7.2;
- Known evidence of chronic interstitial infiltration at imaging;
- Known hospitalization within the past six months for respiratory failure ($PaCO_2$ > 50 mmHg or PaO_2 < 55 mmHg, or oxygen saturation <88% on FiO_2 = 0.21);
- Known chronic vascular disease resulting in severe exercise restriction (i.e. unable to perform household duties);
- Known secondary polycythemia, severe pulmonary hypertension, or ventilator dependency;
- Known vasculitis with diffuse alveolar hemorrhage;.
- Pre-existing renal failure on hemodialysis or peritoneal dialysis requiring renal replacement therapy;
- Extracorporeal membrane oxygenation (ECMO);
- Immunosuppressive treatment;
- Patient in trials for COVID-19 within 30 days before;
- Unstable hemodynamics in the preceding 4 hours (MAP < 65 mmHg, or SAP < 90 mmHg, DAP < 60 mmHg, and/or vasoactive agents required);

- Hyperkalemia , i.e. serum K^+ levels > 5.0 mmol/L, or hyponatremia, i.e. serum Na^+ levels < 130 mmol/L;
- Severe active bleeding;
- Any other uncontrolled comorbidities that increase the risks associated with the study drug administration, as assessed by the medical expert team.

Concomitant treatments

Patients will be allowed to continue their treatment for pre-existing conditions, including ACE-inhibitors, angiotensin II AT1 receptor blockers (ARB).[19–21] Since mineralocorticoid receptor antagonists (MRA) act via eNaC, they will be allowed; however, utmost attention will be given to detect the onset of hyperkalemia, which if occurring will lead to their immediate withdrawal and use of polystyrene sulfonate.

Given the anticoagulant properties of nafamostat, anticoagulants will not be allowed. Antiplatelet drugs, if needed, and on-going treatments with antiviral and anti-inflammatory drugs, if ongoing, will be allowed.

Objectives - Study endpoints

Primary endpoint

Primary endpoint is the time-to-clinical improvement, defined as the time from randomization to an improvement of two points (from the status at randomization) on a seven category ordinal scale. This endpoint was used in a previous study on COVID-19[14] and was recommended by the WHO R&D Blueprint expert group.[22] The seven-category ordinal scale is shown in Table 1.

Secondary clinical endpoints

The following secondary end-points, which were found to bear prognostic information in a retrospective study on clinical course of COVID-19 by Zhou et al.,[12] will be assessed:

1) Rate of responders, defined as patients showing improvement of two points in seven-category ordinal scale at day 7, 10 ,14, 21 and 28.
2) Proportion of patients who progressed to critical illness/death.
3) Change in pO2/FiO2 ratio over time.
4) Change Sequential organ failure assessment score (SOFA score) over time.
5) Improvement at imaging, as assessed at the chest CT (in the participating hospitals a special track has been reserved to the COVID-19 patients).
6) Duration of hospitalization in survivors.
7) Number of patients who require mechanical ventilation and duration of mechanical ventilation
8) Proportion of patients who develop arrhythmia, or myocardial infarction, or other cardiovascular disease not present at the baseline.

Secondary biomarker endpoints:

Changes in relevant biomarkers over time:

- Coagulation and disseminated intravascular coagulation (DIC):
    - PT-INR, aPTT, fibrinogen, D-Dimer and fibrinogen degradation products (FDP), factor XII, X, VII and II activity
    - Plasminogen activator inhibitor type-1 (PAI-1);
- Renin-angiotensin-aldosterone system:
    - Circulating plasma levels of angiotensin peptides (angiotensin 1-8, angiotensin 1-7, angiotensin 1-5), and angiotensin-converting enzyme-1 (ACE-1) and angiotensin-converting enzyme 2 (ACE-2) activity,
    - Direct renin concentration,
    - Plasma aldosterone concentration;
- Infection/inflammation/tissue damage:

- Measurement of viral RNA titer over time and area under-the-curve (AUC)
- Interleukin 6 (IL-6), Soluble tumor necrosis factor receptor type II (sTNFrII),
- Tumor necrosis factor-α (TNF-α),
- Soluble receptor for advanced glycation end products (sRAGE) and Surfactant Protein-D (markers of lung injury),
- AST, ALT,
- High-sensitivity cardiac troponin I,
- C-reactive protein and procalcitonin;
- Serum ferritin, lactate dehydrogenase,

- Endothelial dysfunction:
  - C-terminal fragment of endothelin-1,
  - Von Willebrand factor (vWF);
- Blood cells and immunity:
  - Lymphocyte and neutrophil counts,
  - Hemoglobin, red blood cells and platelet count,
  - IgM and IgG for the Sars-Cov-2 antigens (S protein, etc) will be assessed both during the in-hospital phase and afterward up to 24 months in order to determine the effect of nafamostat on acquired immunity.
- Renal function:
  - Urea, creatinine, eGFR (CKD-Epi),
  - Serum levels of Na^+ and K^+,
  - 24-h urinary excretion of Na^+ and K^+,
  - 24h urine albumin excretion (albumin/g creatinine)

Safety endpoints:

- Adverse events (fatal and non-fatal) occurring during treatment,

- Premature discontinuation of treatment due to adverse events,
- Hyperkalemia defined as S-K+ $\geq$ 5.0 mmol/L, or hyponatremia defined as Na^{+}< 130.0 mmol/L.
- Hemorrhages.

Methods:

Biochemical markers will be measured in an ISO9002-certified laboratory except to the angiotensin peptides that will measured at Attoquant (Attoquant Vienna, A), as reported.[23]

Randomization

To enforce control over bias, randomization will be performed using a permuted block randomization sequence with stratification.[24] Strata will be defined by the cross-combination of use of oxygen therapy (nasal duct, mask, etc.) and ongoing treatment with inhibitors of the renin-angiotensin-aldosterone system, as these drugs have been suggested to affect outcomes.[19,20,25]

Source of drug. Nafamostat will be provided by Kyoso Mirai Pharma.

Statistics

The primary endpoint for this study is the time to a 2-point improvement in a seven-category ordinal scale. Patients who die will be censored at day 28.

The primary endpoint parameter will be compared between the treatment groups using a Log-Rank test. The estimate of the hazard ratio and corresponding 95% confidence interval (CI) will be provided using a Cox proportional hazards (CPH) model including treatment. Kaplan-Meier method will be used to estimate the median time to 2-point improvement and its CI.

The secondary efficacy endpoints will be analyzed using different applicable methods to examine differences between groups. The safety endpoints will be analyzed using descriptive statistics.

Biomarker endpoints will be analyzed using descriptive statistics. Their association with the primary endpoints will be also analyzed using applicable models in the class of Generalized Linear Models (GLM).

Sample size

The study is designed as an event-driven group-sequential parallel-arm trials,[26] with two interim analyses, with non-binding formal control for futility. The primary objective of the study is to detect a statistically significant difference in time to 2-point improvement of the seven-category ordinal scale for the nafamostat arm relative to the control arm, using the classical Lachin design.[27] As the divergence between cases following a non-fatal vs a fatal clinical course started to occur on average after 7-10 days , it is anticipated that the median time for starting detecting a difference between the nafamostat group and the placebo group will fall in between 7 to 10 days, with a hazard ratio between the treatment arms of 0.70.[12] We also estimated that 200 events are needed at the final analysis, assuming a censoring time at 30 days, an overall study duration of 6 months and a uniform overall accrual time of 4 months, with a symmetric two-sided alpha level of 0.05 and a power of 0.80. This number of events corresponds to an expected total sample size of 256 patients. Two equally-spaced interim analyses have been foreseen based on the number of events observed, using a O'Brien-Fleming boundary for the alpha-spending function (Figure 1). The first analysis will be performed after 67 events will be observed overall in the study; the second at 133 events (**Errore. L'origine riferimento non è stata trovata.**). At each interim analysis, the Z-statistics will be computed and compared with the corresponding boundary. If the Z-statistics crosses the upper-boundary, the efficacy of namafostat is proven at that interim. If the Z-statistics crosses the lower-boundary, the efficacy of namafostat is considered not be evaluable with the current study, and the trial will stop for futility; otherwise the study will continue until the total number of events will be observed. At each interim evaluation, the hazard ratios between control and namafostat

group will be estimated to describe the strength of the effect that should be observed if stopping occurs, both for proven superiority or for futility (Figure 1). Hence, futility analyses will be performed at the time of interim analysis. Computations have been performed using the R System [28] and the gsDesign libraries.[29]

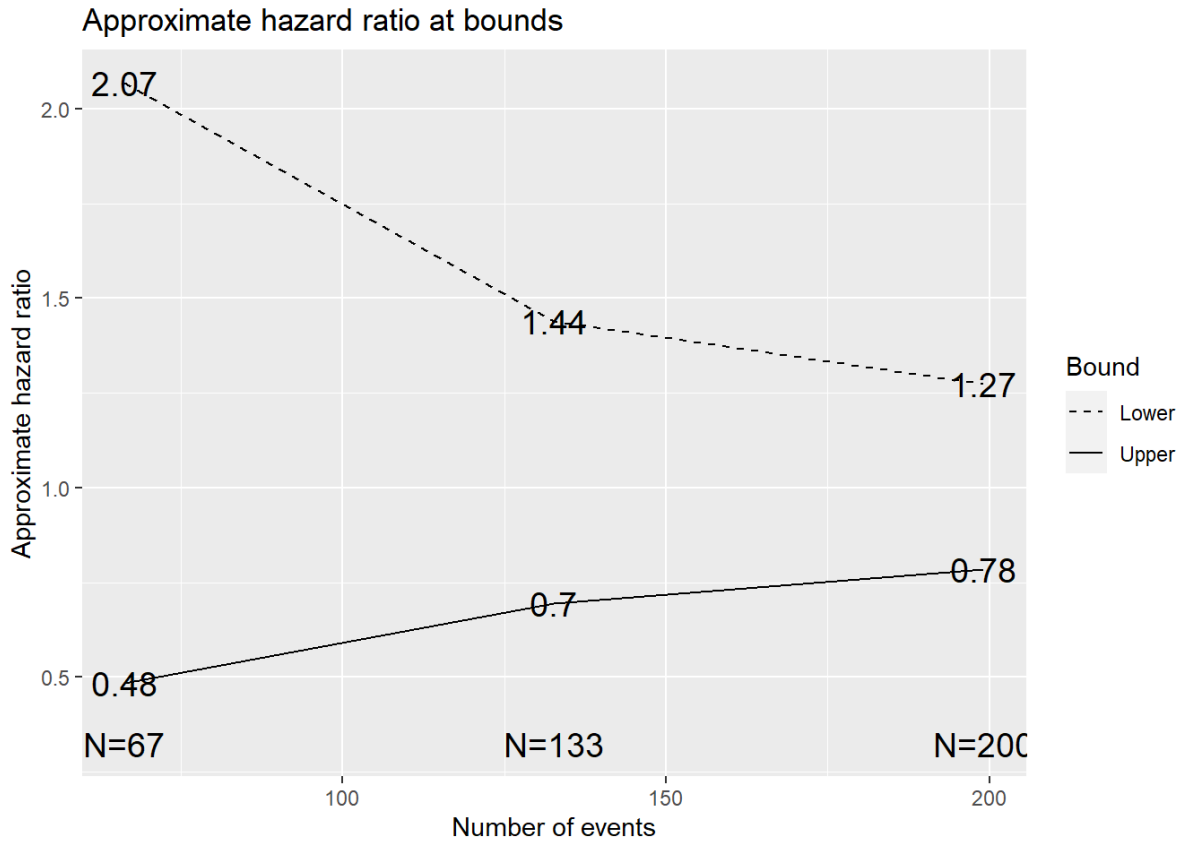


Figure 1. Estimated hazard ratios at each interim that should be observed if stopping occurs, based on O'Brien-Fleming boundaries, and plotted against the number of events in correspondence to each interim. Lower line represents effects at boundary for superiority, upper line represents the effects at boundary for futility.

Expected results

Nafamostat mesylate is already approved for clinical use and its well characterized pharmacodynamic profile makes it particularly appealing as an antiviral drug capable of preventing the spread of SARS-Cov-2 throughout the lung. Moreover, its anticoagulant and mucolytic properties, concur to render it even more attracting considering that pulmonary embolism,[11] disseminated intravascular coagulation, and superinfections are well documented

features of COVID-19.[12]. Moreover, a good deal of experimental data indicate that nafamostat is far more potent than camostat, another TMPRSS2 inhibitor, which was shown to effectively prevented the entry of SARS-Cov-2 into cells.[6]

Besides being well founded from the scientific standpoint, this study has been planned to be adequately powered from the statistical standpoint. Hence, it is well fit to demonstrate a clinically important difference in outcome between the nafamostat and the placebo arm within a limited period of time. We expect that this will allow to rapidly identify if TMPRSS2 inhibition with nafamostat mesylate is a valuable strategy to decrease the hospital stay and lower the number of COVID-19 patients who need to be admitted to the intensive care units.

Discussion

The pandemics of Covid-19 (Coronavirus disease 2019) from the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2) has infected more than a million and killing more than 50,000 people worldwide, about one fourth of them in Italy, as of April 2nd 2020. This calls for urgent deployment of effective therapeutic strategies. The estimated 79% infection rate from undocumented cases in Covid-19 (corona virus disease-19) patients,[30] and the high lethality of SARS-CoV-2 infections, along with its enormous socio-economic impact and fears of renewed outbreaks, make it paramount to develop mechanistically-based treatment strategies in order to effectively prevent need for admission to the intensive care units. The latter, which were not sized to cope with such a planetary emergency, represent the bottleneck that can preclude survival to several infected patients.

While waiting for vaccines, novel drugs, and unambiguous proofs of efficacy of strategies exploiting use of available antiviral drugs, and agents as chloroquine and tocilizumab,[31] it urgent and quite reasonable to investigate the usefulness of drugs that are already available and known to be safe, albeit used for altogether different indications thus far.

Notwithstanding a long-standing experience for clinical use of both camostat and nafamostat mesylate in Japan, to our knowledge, no randomized clinical trial has involved such TMPRSS2 inhibitors in COVID-19 patients to date. Nafamostat is being used for several clinical indications, including treatment of chronic pancreatitis, disseminated intravascular coagulation,[32] and prevention of post-ERCP pancreatitis.[33] On the whole, it has proven to be extremely well tolerated and safe even in frail patients as those submitted to extracorporeal membrane oxygenation[9] and hemodialysis because of chronic renal failure, where it is being used to prevent clotting during extracorporeal circulation,[15] even in patients with high risk of hemorrhage or active bleeding.[34]

Of note, nafamostat was shown to inhibit several proteases at sub-micromolar concentrations,[35] including several coagulation factors, but particularly factor VIIa,[36] which, upon binding the tissue factor released by the damaged lung in COVID-19, activates the extrinsic coagulation pathway leading to disseminated intravascular coagulation and pulmonary embolism. Accordingly, this action renders nafamostat particularly appealing in COVID-19.

Moreover, nafamostat mesylate was shown to protect the lung from radiographic contrast medium in the rat [37] and to be 10^4-fold more potent than camostat in inhibiting proteases in human bronchial epithelial cells.[7]

As mentioned, it has been approved by the European Medicines Agency (EMA) for use in children with cystic fibrosis owing to its inhibitory action on the eNaC. Because of this effect, it was reported to raise serum potassium levels and occasionally cause hyperkalemia.[38] Hence, when designing this study attention has been paid to monitor the onset of this potential complication and use of potassium-lowering drugs, as ion-exchange resins, will be exploited in case of hyperkalemia. Moreover, since mineralocorticoid receptor antagonists act via eNaC, attention will be given also to concomitant use of such drugs.

Conclusions and perspectives

Considering the current spread of COVID-19 in Australia and New Zealand in spite of the hot season the SARS-CoV-2 – unlike other *Coronavidiae* – appears to be resistant to higher temperatures. We therefore might have to live with Covid-19 longer than with SARS-CoV, and it cannot be excluded that SARS-CoV-2 will become endemic in the human population. The current containment policies are buying us the time that is needed for developing effective preventive and therapeutic strategies. In the meantime, at this stage it is urgent to start randomized clinical trials using hard endpoints, such as planned in this study. The sooner such trials are initiated, the better it will be, as their results might enable us to reduce the fatal clinical consequences, mainly sepsis, ARDS, and multiorgan failure, of SARS-CoV-2.

Acknowledgements: none.

Competing interests: none.

References

1. Yamamoto M, Matsuyama S, Li X, Takeda M, Kawaguchi Y, Inoue J. 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(11):6532–9.
2. Xu X, Chen P, Wang J, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 2020;63(3):457–60.
3. Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
4. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203(2):631–7.
5. Yan R, Zhang Y, Guo Y, Xia L, Zhou Q. Structural basis for the recognition of the 2019-

nCoV by human ACE2. bioRxiv [Internet] 2020;2762(March):2020.02.19.956946. Available from: https://www.biorxiv.org/content/10.1101/2020.02.19.956946v1

6. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor Article SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell [Internet] 2020;181:1–10. Available from: https://doi.org/10.1016/j.cell.2020.02.052

7. Nimishakavi S, Raymond WW, Gruenert DC, Caughey GH. Divergent inhibitor susceptibility among airway lumen-accessible tryptic proteases. PLoS One [Internet] 2015;10(10):1–17. Available from: http://dx.doi.org/10.1371/journal.pone.0141169

8. Ota S, Hara Y, Kanoh S, Shinoda M, Kawano S. Respiratory Medicine Case Reports Acute eosinophilic pneumonia caused by camostat mesilate : The first case report. Respir Med Case Reports [Internet] 2016;19:21–3. Available from: http://dx.doi.org/10.1016/j.rmcr.2016.06.005

9. Han SJ, Kim HS, Kim K, et al. Use of nafamostat mesilate as an anticoagulant during extracorporeal membrane oxygenation. J Korean Med Sci 2011;26(7):945–50.

10. Asakura H, Ogawa H. Potential of Heparin and Nafamostat Combination Therapy for COVID-19. J Thromb Haemost [Internet] 2020;Available from: http://www.ncbi.nlm.nih.gov/pubmed/32302456

11. Xie Y, Wang X, Yang P, Zhang S. COVID-19 Complicated by Acute Pulmonary Embolism. Radiol Cardiothorac Imaging [Internet] 2020;2(2):e200067. Available from: http://pubs.rsna.org/doi/10.1148/ryct.2020200067

12. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet [Internet] 2020;6736(20):1–9. Available from: http://dx.doi.org/10.1016/S0140-6736(20)30566-3

13. Schulz KF, Altman DG, Moher D, Group C. CONSORT 2010 Statement : Updated Guidelines for Reporting Parallel Group Randomized Trials OF TO. Ann Intern Med 2013;1996(14).

14. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med [Internet] 2020;Available from:

http://www.ncbi.nlm.nih.gov/pubmed/32187464

15. Maruyama Y, Yoshida H, Uchino S, et al. Nafamostat mesilate as an anticoagulant during continuous veno-venous hemodialysis: A three-year retrospective cohort study. Int J Artif Organs 2011;34(7):571–6.

16. Rossi GP, Barisa M, Allolio B, et al. The Adrenal Vein Sampling International Study (AVIS) for identifying the major subtypes of primary aldosteronism. J Clin Endocrinol Metab 2012;97(5):1606–14.

17. Rossi GP, Rossitto G, Amar L T, Rossi GP, Rossitto G, Amar L AM. The Outcomes of Subtyped Primary Aldosteronism Patients In the AVIS-2 Study. submitted 2020;

18. Cesari M, Ceolotto G, Rossitto G, Maiolino G, Seccia TM, Rossi GP. The Intra-Procedural Cortisol Assay During Adrenal Vein Sampling : Rationale and Design of a Randomized Study (I-Padua). High Blood Press Cardiovasc Prev 2017;24(2):167–70.

19. Vaduganathan M, Vardeny O, Michel T, Mcmurray J V., Pfeffer MA, Solomon SD. Renin – Angiotensin – Aldosterone System Inhibitors in Patients with Covid-19. New Engl J Med 2020;1-7 DOI: 10.1056/NEJMsr2005760.

20. Rossi GP, Sanga V, Barton M. Potential harmful effects of discontinuing ACE-inhibitors and ARBs in COVID-19 patients. Elife 2020;9_e57278:1–8.

21. Zhang P, Zhu L, Cai J, et al. Association of Inpatient Use of Angiotensin Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers with Mortality Among Patients With Hypertension Hospitalized With COVID-19. Circ Res [Internet] 2020;CIRCRESAHA.120.317134. Available from: https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.120.317134

22. World Health Organization. WHO R&D Blueprint Informal consultation on prioritization of candidate therapeutic agents for use in novel coronavirus 2019 infection. Geneva: 2020.

23. Vanderriele P, Caroccia B, Seccia TM, et al. The angiotensin type 2 receptor in the human adrenocortical zona glomerulosa and in aldosterone-producing adenoma: low expression and no functional role. Clin Sci 2018;132(6):627–40.

24. Broglio K. Randomization in clinical trials: Permuted blocks and stratification. JAMA - J Am Med Assoc 2018;319(21):2223–4.

25. Zhang P, Zhu L, Cai J, et al. Association of Inpatient Use of Angiotensin Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers with Mortality Among Patients With Hypertension Hospitalized With COVID-19. Circ Res 2020;CIRCRESAHA.120.317134.

26. Lesaffre E. "The Randomized Controlled Trial: Methodological Perspectives." In: Understanding Evidence-Based Rheumatology. 2014. p. 159-178.

27. Lachin JM, Foulkes. MA. Evaluation of sample size and power for analyses of survival with allowance for nonuniform patient entry, losses to follow-up, noncompliance, and stratification. Biometrics 1986;42:507–19.

28. R_Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. [Internet]. 2020;Available from: https://www.r-project.org/.

29. Keaven Anderson K. gsDesign: Group Sequential Design. 30-1, Packag version Https//CRANR-project.org/package=gsDesign 2016;

30. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (COVID-19). Science 2020;3221(March 16):Published online ahead of print DOI: 10.1126/scien.

31. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther [Internet] 2020;14(1):58–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32147628

32. Minakata D, Fujiwara S ichiro, Ikeda T, et al. Comparison of gabexate mesilate and nafamostat mesilate for disseminated intravascular coagulation associated with hematological malignancies. Int J Hematol [Internet] 2019;109(2):141–6. Available from: http://dx.doi.org/10.1007/s12185-018-02567-w

33. Yu G, Li S, Wan R, Wang X, Hu G. Nafamostat mesilate for prevention of post-ERCP pancreatitis. Pancreas 2015;44(4):561–9.

34. Choi JY, Kang YJ, Jang HM, et al. Nafamostat mesilate as an anticoagulant during continuous renal replacement therapy in patients with high bleeding risk a randomized clinical trial. Med (United States) 2015;94(52):1–7.

35. Aoyama T, Ino Y, Ozeki M, et al. Pharmacological Studies of FUT-175, Nafamstat Mesilate I. Inhibition of Protease Activity in in Vitro and in Vivo Experiments. Jpn J Pharmacol 1984;35(3):203–27.

36. Okajima K, Uchiba M, Murakami K. Nafamostat Mesilate. Cardiovasc Drug Rev 1995;13(1):51–65.

37. Sendo T, Itoh Y, Goromaru T, et al. A potent tryptase inhibitor nafamostat mesilate dramatically suppressed pulmonary dysfunction induced in rats by a radiographic contrast medium. Br J Pharmacol 2003;138(5):959–67.

38. Muto S, Imai M, Asano Y. Mechanisms of hyperkalemia caused by nafamostat mesilate. Gen Pharmacol 1995;26(8):1627–32.

Table 1. Seven-category ordinal scale

Score	Descriptor
1	Not hospitalized with resumption of normal activities
2	Not hospitalized, but unable to resume normal activities
3	Hospitalized, not requiring supplemental oxygen
4	Hospitalized, requiring supplemental oxygen
5	Hospitalized, requiring nasal high-flow oxygen therapy, noninvasive mechanical ventilation, or both
6	Hospitalized, requiring ECMO, invasive mechanical ventilation, or both
7	Death

Table 2. Flow-chart of the study

Task	Enrolment	Randomization	Treatment										
			Days										
	-1	0	1	2	3	4	5	6	7	10	14	21	28
Informed consent	X												
Inclusion/exclusion criteria	X												
Demographics[a]	X												
Clinical history	X												
Randomization		X											
Seven-category ordinal scale	X	X	X	X	X	X	X	X	X	X	X	X	X
SARS-CoV-2 nucleic acid detection	X								X				X
Vital signs & clinical examination	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]
Lab tests	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]
ECG	X			X					X		X		X
Chest X-ray	X								X				X
SOFA score	X	X	X	X	X	X	X	X	X	X	X	X	X
Pneumonia Severity Index (PSI)	X	X	X		X		X		X	X	X	X	X
CURB65 score	X	X	X		X		X		X		X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X

a: Demographics as in WHO Global COVID-19 Clinical Platform (Supplemental Material).
b: Measurement of temperature, heart rate, respiratory rate, systolic and diastolic blood pressure, oxygen saturation.
c: see Table 2 Laboratory tests.

Table 3. Laboratory tests

Test	Enrolment	Randomization	Treatment										
	Days												
	-1	0	1	2	3	4	5	6	7	10	14	21	28
Angiotensin peptides	X								X				X
Direct renin concentration and plasma aldosterone concentration	X								X				X
Angiotensin-converting enzyme (ACE)	X								X				X
(ACE2	X								X				X
Interleukin 6 (IL-6),	X			X		X			X				X
Soluble tumor necrosis factor receptor type II (sTNFrII)	X			X		X			X				X
Plasminogen activator inhibitor type-1 (PAI-1)	X			X		X			X				X
Tumor necrosis factor-α (TNF-α)	X			X		X			X				X
Soluble receptor for advanced glycation end products (sRAGE) and Surfactant Protein-D	X			X		X			X				X
Endothelin-1	X			X		X			X				X
Lymphocyte and neutrophil counts	X	X	X	X	X	X	X	X	X	X	X	X	X
SARS-Cov2 IgM/IgG***	X	X				X			X		X	X	X

Haemoglobin and red blood cells	X	X						X	X	X	X	X	X
Platelet counts	X	X	X	X	X	X	X	X	X	X	X	X	X
PT-INR, aPTT, fibrinogen, D-Dimer and fibrinogen degradation products (FDP)	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum ferritin	X			X		X			X				X
Lactate dehydrogenase	X			X		X			X				X
C-reactive protein and procalcitonin	X			X		X			X				X
Urea, creatinine, eGFR	X	X	X	X	X	X	X	X	X	X	X	X	X
S-Na+, S-K+,	X	X	X	X	X	X	X	X	X	X	X	X	X
U-Na+,US-K+,	X	X	X	X	X	X	X	X	X	X	X	X	X
AST ALT	X	X	X	X	X	X	X	X	X	X	X	X	X
High-sensitivity cardiac troponin*	X			X		X			X				X
Viral RNA titer	X			X									
Pregnancy Test**	X												
Urinalysis	X			X		X			X				X

* At any time if patient symptomatic for chest pain or other symptoms suggesting myocardial ischemia

If positive, do not enroll the patient. * These variables will be assessed also after discharge at 3, 6, 12 and 24 months.

Table 4- Details of the group-sequential design adopted for the study. P(Cross) is the probability of crossing the bound at or before the given analysis under the assumed hazard ratio (HR). P(cross) if HR=0.7 for Futility is <0.001 at each interim.

Analysis	N events	Z (Efficacy)	Z (Futility)	Nominal P	Alpha spent	HR at bound for Efficacy	HR at bound for Futility	P(cross) if HR=1 for Efficacy or Futility	P(cross) if HR=0.7 for Efficacy
1	67 (33%)	2.96	-2.96	0.0015	0.0015	0.4833	2,069	0.0015	0.0660
2	133 (67%)	2.09	-2.09	0.0181	0.0172	0.6952	1.438	0.0187	0.4879
3	200 (100%	1.71	-1.71	0.0437	0.0313	0.7848	1.274	0.0500	0.8000
Total					0.0500				