

TMPRSS2: Potential Biomarker for COVID-19 Outcomes

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Coronavirus disease 2019 (COVID-19) clinical data has so far shown that the mortality rate for men is higher than for women. This disparity is observed worldwide and across different ethnic/racial groups (Table 1). Early reports from Italy and Germany show that while infection rates are similar between sexes, nearly 70% and 65%, respectively, of deaths are males. In New York City, an epicenter of the US outbreak, 54% of those infected are men, yet men account for 63% of deaths. Epidemiologic data from the previous coronavirus infections, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), also indicated sex-based differences in disease susceptibility and outcomes. This discrepancy was attributed to many factors, including smoking, immune differences, and other comorbidities. An initial report released by the Centers for Disease Control and Prevention (CDC) on population-based surveillance sampled across 14 states, representing 10% of the US population, has indicated that age and comorbidities are associated with increased hospitalization rates of patients with COVID-19.¹ The data on sex also suggest sexual dimorphism consistent with reports from other countries (Table 1). The preliminary data on race suggest that minority populations may be disproportionately impacted by the coronavirus, where blacks contributed to 33% of the hospitalizations despite representing only 18% of the sampled population. As more data become available, correlations between race and disease severity can be interrogated more thoroughly, including the role of socioeconomic factors on influencing this disparity. Investigations into the genetic and molecular differences between women and men are warranted to identify relevant biomarkers for disease susceptibility and outcomes. Based on data from literature, we propose a novel mechanism of the observed sex differences in clinical outcomes in patients and identify a role for

the transmembrane protease serine 2 (TMPRSS2) as a contributing factor to the more severe outcomes noted for COVID-19.

TMPRSS2 Expression as a Biomarker of Clinical Outcomes

The angiotensin I-converting enzyme 2 (ACE2) and TMPRSS2 have been implicated in influenza and SARS-corona virus (CoV) infection as well as for SARS-CoV-2 in mediating viral entry into the host cell.² Both genes mediate sex-specific effects with ACE2, located on the X chromosome and TMPRSS2 regulated by androgen (located on 21q22.3). While ACE2 is the main receptor for the spike protein for both viruses, its expression and gene polymorphisms did not influence sex-specific effects or outcomes for SARS³ or COVID-19 (based on 2 recent preprint studies published on medRxiv that have yet to be peer-reviewed at the time of writing).^{4,5}

TMPRSS2 is an androgen-responsive serine protease that cleaves SARS-CoV-2 spike protein, facili-

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Table 1. Incidence of Mortality in COVID-19 Patients^a

Location	Sex	Incidence, %	Death, %	
North America	United States ^b	Female	46 ^g	41
		Male	54 ^g	59
	Chicago ^c	Female	49	41
		Male	50	59
	New York ^d	Female	47	38
		Male	53	62
	Washington State ^e	Female	52	43
		Male	45	57
	Michigan ^f	Female	54	43
		Male	45	57
	Canada	Female	54	48
		Male	46	58
	Mexico	Female	42	30
		Male	58	70
Europe	Italy	Female	48	33
		Male	52	67
	France	Female	53	39
		Male	47	61
	Germany	Female	51	41
		Male	49	59
	Denmark	Female	56	39
		Male	44	61
	Ireland	Female	55	41
		Male	45	59
	Spain	Female	51	37
		Male	49	63
	Switzerland	Female	53	39
		Male	47	61
	The Netherlands	Female	58	39
		Male	42	61
Asia	South Korea	Female	60	48
		Male	40	52
	Philippines	Female	45	31
		Male	55	69
	China	Female	49	36
		Male	51	64
South America	Ecuador	Female	42	30
		Male	58	70
	Columbia	Female	49	38
		Male	51	62
	Peru	Female	42	28
		Male	58	72
Australia	Female	49	40	
	Male	51	60	

^aData from <https://globalhealth5050.org/covid19/> and the websites in notes b-g (accessed on April 16, 2020).

^b<https://www.cdc.gov/nchs/nvss/vsrr/COVID19/>

^c<https://www.chicago.gov/city/en/sites/covid-19/home/latest-data.html>

^d<https://www1.nyc.gov/assets/doh/downloads/pdf/imm/covid-19-daily-data-summary-deaths-04072020-1.pdf> and

<https://www1.nyc.gov/assets/doh/downloads/pdf/imm/covid-19-daily-data-summary-04072020-1.pdf>

^e<https://www.doh.wa.gov/emergencies/coronavirus>

^fhttps://www.michigan.gov/coronavirus/0,9753,7-406-98163_98173--,00.html

^g<https://www.cdc.gov/mmwr/volumes/69/wr/mm6915e3.htm> (data from hospitalized patients in 14 states).

tating viral entry and activation.² It is expressed in many tissues outside of the lungs⁶ and is well known for its predominant expression in the prostate epithelium and its role in prostate carcinogenesis.^{7,8} The androgen-regulated promoter of *TMPRSS2* forms a fusion gene with coding regions of the proto-oncogenic ETS transcription factor family members, which is strongly associated with prostate cancer and regulates many biological processes.⁹ *TMPRSS2* is also expressed on the cardiac endothelium, kidney, and digestive tract, suggesting that these may be important target organs for SARS-CoV-2 infection.⁶ Indeed, the clinical manifestations of COVID-19 include not only complications from acute myocardial injury, elevated liver enzymes, and acute kidney injury in patients presenting to hospitals, but also gastrointestinal symptoms in community patients experiencing milder forms of the disease.^{10,11} Because *TMPRSS2* is also expressed on microvascular endothelial cells that line blood vessels,⁶ viral infection by SARS-CoV-2 may cause endothelial dysfunction, which can lead to thrombosis and associated complications. These data present a rationale for the role of *TMPRSS2* in clinically observed complications in patients with severe disease.

***TMPRSS2* Single-Nucleotide Polymorphisms as Biomarkers of Disease Outcomes**

Studies into *TMPRSS2* single-nucleotide polymorphisms (SNPs) have been conducted in various diseases. In breast cancer, the minor allele for rs2276205 (A>G) was associated with increased survival, potentially due to sensitization to tamoxifen.¹² In prostate cancer, the most studied disease in relation to *TMPRSS2*, rs12329760 (C>T) was associated with cancer in men with a family history of prostate cancer, multiple copies of the *ERG* gene fusion, and in whites with a doubled time to cancer diagnosis.^{13,14} Rs2070788 (G>A) and rs383510 (T>C) were associated with severe H1N1, H7N9, and increased expression of *TMPRSS2* in the lungs.¹⁵ Most recently, a preprint study comparing genetic variants in *TMPRSS2* among Italians, Europeans, and East Asians, showed allele frequency differences in rs12329760 and 2 distinct eQTL haplotypes between Italians and East Asians.⁵ The study proposed that *TMPRSS2* could possibly be a candidate gene that contributes to the COVID-19 epidemiologic data seen in the Italian population, with higher death rates and differences in severity among sexes compared to East Asians. Interestingly, Asian men have a decreased incidence and mortality of prostate cancer and a lower frequency of the *TMPRSS2:ERG* fusion gene.^{16,17} We may never know the true incidence rates for COVID-19; however, looking at current data, Europe and the

United States have overtaken China and eastern Asia as the epicenters of the pandemic. Accurate infection rates may demonstrate that East Asians are less susceptible to severe disease outcomes.

TMPRSS2 Inhibitors, Rationale for Drug Combinations, and Clinical Biomarkers of Response

TMPRSS2:ERG fusions can upregulate genes related to the nuclear factor- κ B pathway including Toll-like receptor-4 and the Notch pathways in prostate cancer.^{18,19} The role of *TMPRSS2* and *TMPRSS2:ERG* fusions in nonprostatic tissue remain to be elucidated, including whether this gene can subsequently activate the Notch signaling cascade in macrophages to augment Toll-like receptor-associated inflammatory responses and release of proinflammatory cytokines, such as tumor necrosis factor- α , interleukin (IL)-1 β , and IL-6.²⁰ Clinically, increases in IL-6 have been linked to severe COVID-19 cases²¹ and have been discussed as a potential biomarker.²² Cytokine release syndrome (CRS) has been noted in some severe patients of COVID-19.²³ Coincidentally, *TMPRSS2*-deficient mice were protective against SARS-CoV infection and showed lower expression levels of cytokines and chemokines, suggesting that *TMPRSS2* may be involved in regulating the production of these inflammatory markers.²⁴ Furthermore, autopsy findings from COVID-19 patients revealed that tissues that had been destroyed by SARS-CoV-2 exhibited characteristics of the so-called primary cytokine storm (induced by viral infection and mainly produced by alveolar macrophages, epithelial cells, and endothelial cells), rather than those observed in secondary cytokine storm (induced by different subsets of activated T lymphocytes in late-stage viral infections or a complication of T-cell-engaging therapies).²⁵ Together with data from the *TMPRSS2* knockout mice, which reveal that *TMPRSS2* contributes to the spread and immunopathology of the virus at primary sites of infection, the evidence further points to an underlying mechanism that may involve endothelial dysfunction, which can lead to thrombi formation that is observed in many cases.²¹ Moreover, because the physiological role of *TMPRSS2* is unknown, what remains to be determined is the role of androgen/androgen signaling and other sex steroids in modulating *TMPRSS2* expression during active infection and/or in driving potential novel fusions with possibly another ETS family member that regulate genes involved in the immune response. Our laboratory and others have demonstrated that antiandrogens (eg, enzalutamide, apalutamide) can decrease *TMPRSS2* expression in

prostate cancer cells.^{26,27} Whether the use of these androgen pathway inhibitors to decrease *TMPRSS2* expression has activity against SARS-CoV infection also remains to be addressed.

There are currently no US Food and Drug Administration-approved treatments for any human CoV infection, and new interventions are likely to require months to years to develop. In the face of a pandemic, drug repurposing, using existing medicines that having already been tested safely in humans, is an emerging strategy that offers a faster approach to identifying an effective COVID-19 treatment. Rigorous testing in double-blinded, randomized, controlled trials with a larger sample size are needed to determine the safety and efficacy of these new drug combinations and to guide clinical decisions. While each repurposed drug individually may not yield a significant overall clinical benefit, carefully combined cocktails could be very effective (eg, HIV), focusing on a multimodality approach. The key is to find the right combination in the case of COVID-19.

In standard drug development, many proposed combinations would usually undergo *in vivo* preclinical testing to provide rationale for the regimen. However, there are currently no robust mouse models that recapitulate the SARS-CoV-2 pathogenesis observed in humans. Due to structural differences in mouse ACE2 compared to human ACE2 (hACE2) proteins, the SARS coronaviruses exhibit poor tropism characteristics for mouse tissues and are inefficient at infecting mice. McCray et al²⁸ successfully developed a hACE2 transgenic mouse strain (K18-hACE2) as a mouse model for SARS. Since SARS-CoV-2 shares similar mechanisms of viral entry with SARS-CoV, this transgenic model could potentially be used to study novel therapeutics for COVID-19. The Jackson Laboratory is currently in the process of reestablishing a new K18-hACE2 transgenic mouse colony. Because SARS-CoV-2 disease severity increases with increasing age, we suggest future drug efficacy testing in CoV aged mouse models to fully recapitulate the age-related increase in pathogenesis observed in humans. In summary, no *in vivo* preclinical data for SARS-CoV-2 are available to justify any of the treatment regimens for combination trials currently under investigation for COVID-19, as an *in vivo* mouse model for SARS-CoV-2 is not yet available, and it would require too much time to test each proposed drug combination in primate models of human disease or use existing models for MERS-CoV and SARS-CoV pathogenesis.

Given the urgency of the COVID-19 pandemic, repurposing existing drugs or antiviral agents approved or in development for treating infections caused by HIV, hepatitis B/C, and influenza are mostly based on therapeutic experience with SARS/MERS. In fact,

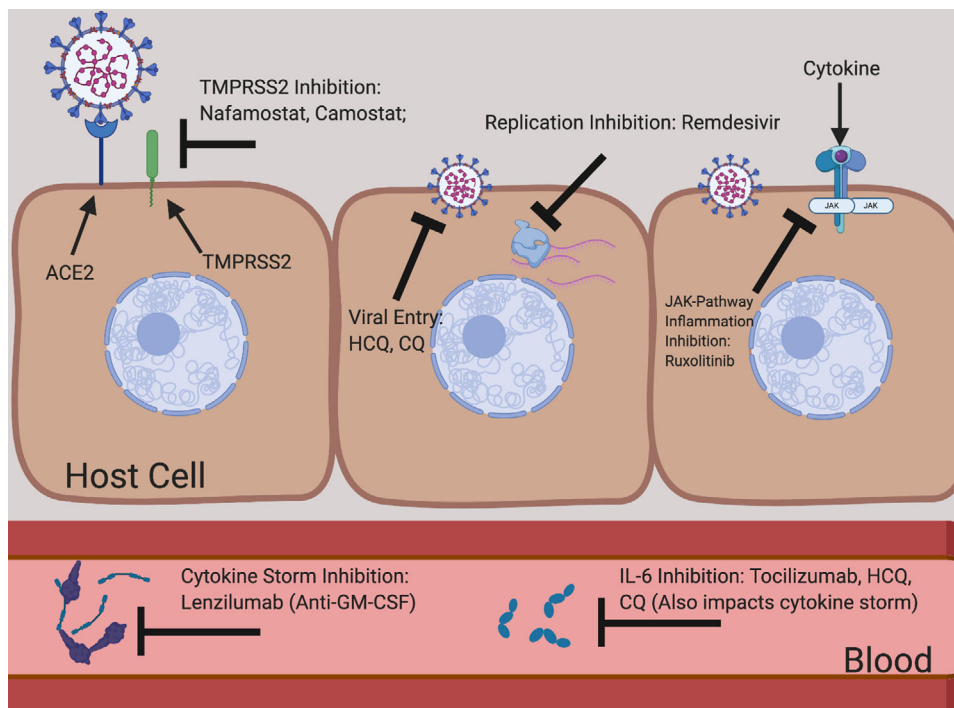


Figure 1. Proposed COVID-19 treatments. Rationale for combination therapy that effectively limits viral entry and replication as well as targets systemic clinical manifestations of viral infection such as cytokine storm. Direct TMPRSS2 inhibition with camostat and nafamostat. Remdesivir, an RNA-dependent RNA polymerase inhibitor with anti-viral activity, targets viral replication post entry in SARS-CoV-2. Hydroxychloroquine (HCQ) and chloroquine (CQ) exhibit broad spectrum effects that include viral inhibition, suppression of multiple cytokines, and vascular protective effects. Some agents target cytokine release such as the anti-IL-6 antibody tocilizumab, the anti-human GM-CSF monoclonal antibody lenzilumab, or the JAK 1/2 inhibitor ruxolitinib. ACE2, angiotensin I-converting enzyme 2; COVID-19, coronavirus disease 2019; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; JAK, Janus kinase; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. Created using BioRender.com.

many of the proposed combination trials registered on the World Health Organization (WHO) website and ClinicalTrials.gov, including the current WHO Solidarity trial, are testing drug combinations based on existing case studies and anecdotal evidence from early treatment approaches used in China, in vitro/in vivo data from testing for SARS/MERS, and histopathological evidence from COVID-19 cases. Many of the proposed treatment combinations are based on demonstrated antiviral activity of the drug(s) in tissue culture coupled with scientific rationale based on proposed mechanisms of action in conjunction with the need for effective therapy against SARS-CoV-2 to justify the need.

There is no doubt that antivirals and supportive treatments are key areas to focus on in treating patients with COVID-19. Data from SARS- and MERS-CoV infections show that the disease process is driven by both virus and host immune response factors. Our proposed treatment combination is based on existing data from in vitro/in vivo studies that have been conducted for SARS-CoV and MERS-CoV for each individual agent as well as clinical features of COVID-19 since current in vivo animal models are not available for SARS-CoV-2. We propose a drug combination therapy

that can effectively limit viral entry and replication as well as target systemic clinical manifestations of viral infection (Figure 1). For example, we propose the rationale for a treatment regimen that includes a TMPRSS2 inhibitor in combination with antivirals and/or inhibitors of CRS. Camostat and nafamostat are TMPRSS2 inhibitors currently approved to treat pancreatitis in Japan. These drugs have been shown to inhibit MERS infection by blocking viral entry, with nafamostat being the more potent of the two.²⁹ Both are being investigated in Japan and other countries for their ability to block SARS-CoV-2 entry, with Japan initiating a clinical trial for nafamostat (which is also a short-acting anticoagulant) for COVID-19-positive patients.³⁰ Direct inhibition of TMPRSS2 using protease inhibitors (nafamostat) or indirectly by downregulating TMPRSS2 expression with androgen pathway inhibitors, as with all potential novel treatments for COVID-19, are promising hypotheses, and it remains to be determined whether this approach can mediate the sex-specific outcomes and severity of outcomes in patients.

Another drug under investigation for COVID-19 is remdesivir, an RNA-dependent RNA polymerase inhibitor with antiviral activity, which has been shown

to act on viral replication after entry in SARS-CoV-2.³¹ While remdesivir is not US Food and Drug Administration approved for any indications, the WHO considered it as a promising drug candidate to treat COVID-19 based on its broad-spectrum activity, in vitro/in vivo data for coronaviruses (SARS-CoV-2, SARS-CoV, and MERS-CoV), and clinical safety data from Ebola virus disease trials. Initial results of the compassionate use of remdesivir in patients with COVID-19 have shown some encouraging results.³² Additionally, there are clinical trials using the antimalarial drugs hydroxychloroquine (HCQ) and chloroquine to treat COVID-19 patients. Many studies have published in vitro data discussing their effects on viral inhibition.^{31,33} Interestingly, antimalarial drugs have been shown to inhibit multiple cytokines including IL-6³⁴ and are speculated to have vascular protective effects to prevent thrombotic complications.³⁵ These data suggests that HCQ and chloroquine may exhibit broad-spectrum effects as antivirals, while simultaneously decreasing a proposed marker of severity and other clinically observed complications. The clinical data for HCQ from early trials for COVID-19 have been mixed, with some studies showing encouraging results and others demonstrating lack of efficacy.³⁶⁻³⁸ This is primarily due to limitations in study methodologies and small patient sample size. We eagerly await the results from larger-scale confirmatory trials for HCQ and remdesivir. Potential inhibitors of cytokine storm have shown some clinical benefit in COVID-19 patients and include agents that target cytokine release such the anti-IL-6 antibody tocilizumab, the anti-human granulocyte macrophage colony-stimulating factor monoclonal antibody lenzilumab, or the Janus kinase 1/2 inhibitor ruxolitinib (with phase III trials initiated or being planned for COVID-19). Sanders et al³⁹ provide a comprehensive review of the recent clinical experience for remdesivir, HCQ, and other therapeutics, including inhibitors of CRS that are currently under investigation as potential COVID-19 treatments.

The CDC clinical guidance currently has listed laboratory markers that are associated with increased illness severity.⁴⁰ These include lymphopenia, neutropenia, elevated serum alanine aminotransferase and aspartate aminotransferase, elevated lactate dehydrogenase, high C-reactive protein, and high ferritin. Additionally, elevated d-dimer and lymphopenia are also associated with mortality. Since high levels of inflammatory markers such as IL-6 have been reported in patients experiencing COVID-19 complications, we suggest using this potential clinical biomarker or any of the above laboratory markers as a guide to monitor patients on drug therapy. COVID-19 treatment will involve a polypharmacy approach; we therefore expect a multitude of adverse effects from these drug combinations. Future

studies will need to address the pharmacogenetics of these therapeutics and potential drug-drug interactions and determine clinical biomarkers of response or in the management of adverse reactions.

Future Studies on *TMPRSS2* as a Predictive Biomarker of COVID-19 Outcomes

Many factors can be used to explain why some are at risk of more serious disease and many are currently being studied. These include socioeconomic status, access to health care, past medical history, and age. There are also many factors that can explain the difference in outcomes in men and women afflicted with COVID-19, and *TMPRSS2* emerges as an interesting candidate. We propose studies examining the differential expression of *TMPRSS2* between male and female patients. Evidence of higher expression in the tissue of males can help inform further studies to correlate this expression to clinical outcomes. Moreover, genotyping studies performed on clinical specimens collected from patients on COVID-19 trials are warranted to further evaluate known SNPs and to find additional functional polymorphisms in the *TMPRSS2* gene. This will aid in understanding the impact of *TMPRSS2* SNPs on disease susceptibility and its correlation with clinical outcomes, which may include assessing the severity of outcomes by organ systems to identify for sexual dimorphism. On a broader scope, multiple institutions have initiated genomic investigations in a project called the COVID Host Genetics Initiative.⁴¹ Underlying genetic polymorphisms present a potential avenue as an actionable biomarker, enabling medical professionals to identify and treat those patients who will need an increased level of care vs those who do not need rigorous medical treatment when resources are scarce. Validated *TMPRSS2* SNPs that are confirmed to be predictive biomarkers can be incorporated in the CDC's current list of clinical biomarkers for disease severity as discussed above. Furthermore, understanding the molecular differences that lead to changes in clinical outcomes, especially clear sexual dimorphisms, can help inform targeted drug therapy research and development. With the uncertainty currently surrounding the development of a vaccine for SARS-CoV-2, the discovery of pharmaceutical interventions is imperative, and understanding the genetics of the disease is equally as important.

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Conflicts of Interest

The authors declare no conflicts of interest.

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