



Commentary

COVID19 therapeutics: Expanding the antiviral arsenal

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Worldwide vaccination will greatly reduce SARS-CoV-2 transmission and severe COVID19. Yet even after mass vaccination, the virus may not be extinguished – infected animal reservoirs will remain and there may be intrusions of the virus back into humans, possibly as variant forms that can circumvent immunizing antiviral antibodies [1]. Inspiring advances in vaccinology and antiviral drug development are taking place to face the challenges of animal virus spillover and possible resurgence of virulence in humans. Adaptable vaccine platforms are in place to protect against variants of concern, and therapeutic regimens are developing to limit early stages of virus growth as well as later life-threatening immunopathological sequelae of infection. The scope of the problem, however, calls for a larger arsenal of vaccines and antiviral agents.

Hoffmann et al. [2; in this issue] are amongst those answering the call. Their works build from decades of research on the mechanisms by which CoVs enter into host cells. CoV-cell entry is a multi-step process in which viral spike proteins first attach to cell receptors and then become “activated” into forms that can catalyze the final essential step in which virus and cell membranes fuse together. Activation requires fusion-catalyzing spike protein fragments that are generated through proteolysis. Host-cell proteases execute the activating proteolytic cleavages, and therefore, inhibitors disabling these host proteases are effective antiviral agents [3]. Yet there are complicating factors in developing protease inhibitors as anti-CoV drugs. During cell entry, CoVs can traverse several cell-surface and endosomal regions before fusing into host membranes, and at each place on the entry pathway there may be several different proteases that can mediate the necessary “activating” scission of spike proteins [4]. Indeed, findings made prior to the COVID19 pandemic made it clear that different members of a relatively large type II transmembrane serine protease (TTSP) family can cleave and activate CoV spikes [5]. Hence the questions: How many different TTSP family members might activate SARS-CoV-2 for virus-cell fusion, and if several can, will a single protease inhibitor block them all and thereby have potential clinical antiviral utility?

Hoffmann et al. addressed these questions in several ways. They established *in vitro* culture systems in which surrogates of SARS-CoV-2 can be evaluated for cell entry, and then supplied these assay platforms with individual TTSP members (termed “TMPRSS” proteases). They found four family members beyond the previously recognized TMPRSS2 [6] conferring susceptibility to virus entry. With this newfound knowledge, they analyzed single-cell transcriptome datasets to determine whether the different TTSPs might be present in human airways, in locations coincident with the ACE2 receptors to which SARS-CoV-2 bind. Several TTSPs were found to be co-expressed with ACE2, and interestingly, each was prevalent in distinct cells of the respiratory tract. These results raise important new questions about the ways that SARS-CoV-2 and other CoVs might adapt to different TTSPs and thereby establish infection and pathogenesis in distinct sites within the pulmonary system – questions that may be addressed in future studies using TTSP-knockout animals and SARS-CoV-2 infection and disease models [7].

Hoffmann et al. then asked whether camostat mesylate, a small-molecule TMPRSS2 inhibitor, could suppress virus entry catalyzed by the other TTSP family members. Put succinctly, camostat effectively prevented all TTSP-activating virus-cell entry. While the conditions used to assess camostat activity involved artificial TMPRSS expression, the results were nevertheless very clear, and they provide a worthy advance toward the antiviral potential of this protease inhibitor. The investigations went forward to address the pharmacologic considerations necessary to promote clinical utility. Camostat mesylate is unstable *in vivo*, rapidly converting to metabolites 4-(4-guanidinobenzoyloxy) phenylacetic acid (GBPA) and then more slowly to 4-guanidinobenzoic acid (GBA). Antiprotease activity of a GBPA derivative was evaluated biochemically and found to block the TMPRSS2 enzyme ~10% as effectively as camostat, with both compounds operating as active-site inhibitors. Finally, both camostat and the GBPA derivative were nearly equal in suppressing authentic SARS-CoV-2 infection into cells derived from human airway epithelia. Overall, the results further support camostat mesylate as a viable treatment option for COVID19.

Camostat mesylate has been approved in Japan for treatment of pancreatitis [8]. The Hoffmann et al. report in this issue combines with several prior works to promote repurposing of camostat and its analog nafamostat to treat COVID19 and other diseases caused by CoVs. Yet there are more steps on the path toward clinical use. There has been limited but insightful evaluation of TTSP inhibitors in mouse models of human SARS-CoV infection [9]. An important next step is to determine whether camostat suppresses disease in recently

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developed SARS-CoV-2 small animal models [7,10]. The animal models may assist in determining the most effective doses and routes of camostat administration, and the post-infection time periods at which camostat must be present in order to reduce virus transmission and disease. It is possible that the therapeutic time window for these drugs is restricted to the onset of acute virus infection, when overt clinical symptoms have not yet appeared. Finally, while Hoffmann et al. convincingly demonstrated that camostat mesylate blocked several TTSPs, there are well known CoV pathways in which entry activation is mediated by proteases other than the TTSPs. These TTSP-independent pathways may not be available to SARS-CoV-2 in human airways and lungs, but if they are accessible at extrapulmonary sites, then drugs other than the TTSP inhibitors will be needed for broad infection control. Combination drug formulations will be central to broad control of SARS-CoV-2 and other CoV-cell entry processes, with biopharmaceuticals and small-molecule drugs targeting both viral spikes and host susceptibility factors to impede several steps in virus-cell entry and thereby reduce infectious disease.

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Declaration of Competing Interests

The author declares no conflict of interest.

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