Targeting TMPRSS2 and Cathepsin B/L together may be synergistic against SARS-CoV-2 infection

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Abstract

The entry of SARS-CoV-2 into target cells requires the activation of its surface spike protein, S, by host proteases. The host serine protease TMPRSS2 and cysteine proteases Cathepsin B/L can activate S, making two independent entry pathways accessible to SARS-CoV-2. Blocking the proteases prevents SARS-CoV-2 entry in vitro. This blockade may be achieved in vivo through ‘repurposing’ existing drugs and offers a potential treatment option for COVID-19, currently in clinical trials. Here, we found, surprisingly, that drugs targeting the two pathways, although independent, could display strong synergy in blocking virus entry. We predicted this synergy first using a mathematical model of SARS-CoV-2 entry and dynamics in vitro. The model considered the two pathways explicitly, let the entry efficiency through a pathway depend on the corresponding protease expression level, which varied across cells, and let inhibitors compromise the efficiency in a dose-dependent manner. We showed, analysing our model, that the synergy was novel and arose from effects of the drugs at both the single cell and the cell population levels. Validating our predictions, we found that available in vitro data on SARS-CoV-2 and SARS-CoV entry displayed this synergy. Exploiting the synergy may improve the deployability of drug combinations targeting host proteases required for SARS-CoV-2 entry.
Introduction

As of April 26, 2020, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had infected a total of over 2.8 million people and caused over 193000 deaths across the world, starting from the first reported infections in Wuhan, China in December, 2019. SARS-CoV-2 is the third of the major coronavirus outbreaks this century, following SARS coronavirus (SARS-CoV) in 2002-03 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012-13. The scale of the current pandemic far exceeds the earlier two and calls for urgent interventions. No approved drugs or vaccines exist for any of these coronaviruses. Massive efforts are ongoing to ‘repurpose’ drugs approved for unrelated conditions that may have an effect on SARS-CoV-2. For instance, the anti-malarial drug hydroxychloroquine, which has shown some activity against SARS-CoV-2, has been approved in some countries, including India, as a prophylactic for high risk groups such as healthcare providers. Several such drugs are under clinical trials, including remdesivir, type I interferons and the HIV-1 drugs lopinavir and ritonavir. Simultaneously, efforts are underway to develop effective vaccines.

An important class of drugs under investigation putatively targets host factors involved in the lifecycle of SARS-CoV-2, which are being identified through exhaustive virus-host interactome maps. Within this class, drugs that block SARS-CoV-2 entry into target cells may be particularly promising, for they would protect cells from becoming infected by the virus and may thus minimize disease. SARS-CoV-2 entry requires the binding of its spike protein, S, to the host cell surface receptor angiotensin-converting enzyme 2 (ACE2). In addition, S must be cleaved by host proteases, as with other coronaviruses and influenza viruses, for successful entry, a step often limiting the zoonotic potential of coronaviruses. In vitro studies have suggested that this cleavage, also termed S protein activation, can be accomplished by the transmembrane serine protease TMPRSS2 and endosomal cysteine proteases Cathepsin B and Cathepsin L. These proteins have also been implicated in the entry of SARS-CoV, to which SARS-CoV-2 is closely related, and have formed the basis for corresponding studies on SARS-CoV-2. (MERS-CoV uses a different receptor, dipeptidyl peptidase 4, but appears to use the same proteases for activation.)
Thus, are key host targets for blocking SARS-CoV-2 entry. ACE2 plays a critical role in regulating several vital parameters, such as blood pressure, and targeting it could come with serious risks. Targeting host proteases appears to have fewer side effects. Indeed, camostat mesylate, a drug that targets TMPRSS2, has been approved for use in the treatment of chronic pancreatitis and postoperative reflux esophagitis in Japan. A human clinical trial has now been initiated to assess the possibility of using camostat mesylate monotherapy for treating SARS-CoV-2 infection (ClinicalTrials.gov ID: NCT04321096). Drugs targeting Cathepsin B/L are under development. Hydroxychloroquine is thought to act against SARS-CoV-2 via several mechanisms, including blocking the endocytic pathway; it suppresses clathrin-mediated virus uptake and also prevents endosomal acidification, the latter required for Cathepsin B/L activity. Human clinical trials with hydroxychloroquine monotherapy have been initiated (e.g., ClinicalTrials.gov ID: NCT04261517). Furthermore, a trial evaluating the efficacy of the hydroxychloroquine and camostat mesylate combination therapy is underway (ClinicalTrials.gov ID: NCT04338906).

The proteases TMPRSS2 and Cathepsin B/L are unrelated and work independently, suggesting that SARS-CoV-2 can enter cells via two independent pathways. Studies on SARS-CoV have shown that TMPRSS2 is expressed on the target cell surface and acts by cleaving S and facilitating the fusion of viral and cell membranes at the target cell surface, whereas Cathepsin B/L are expressed in endosomes and act after the virus has been endocytosed, facilitating the fusion of viral and endosomal membranes. It appears, thus, that blocking either TMPRSS2 or Cathepsin B/L may be insufficient to block virus entry; the virus could continue to enter cells via the pathway that remains unblocked. Indeed, in vitro studies have shown, both with SARS-CoV and SARS-CoV-2, that simultaneous targeting of both TMPRSS2 and Cathepsin B/L is required for fully stopping virus entry. The pathways have not been studied in SARS-CoV-2 infection in vivo.

Given the clinical trials underway to block SARS-CoV-2 entry, we were interested in assessing whether combination therapy that simultaneously targeted both the entry pathways would be advantageous over therapies targeting the pathways individually. To this end, we developed a mathematical model of SARS-CoV-2 dynamics under treatment with a TMPRSS2 inhibitor, a Cathepsin B/L (or endosomal pathway) inhibitor, or both. We explicitly accounted for the dependence of viral entry efficiency via the two pathways.
on the expression levels of the two proteases and the effects of the drugs in suppressing the respective efficiencies in a dose-dependent manner. Our model predicted that targeting the two pathways simultaneously was likely not only to be efficacious but also synergistic.

Synergy between drugs implies that their combined effect is more than their individual effects added together.\textsuperscript{31–34} Synergistic drug combinations are preferable because they allow the realization of the desired efficacy with lower net drug exposure, thus reducing toxicity. Conversely, at dosages limited by toxicities, synergistic drug combinations achieve higher efficiencies than their non-synergistic counterparts. Synergy is thought to arise typically as the result of downstream interactions between the steps/pathways targeted by the drugs.\textsuperscript{31–34} The synergy we predict here is thus surprising because the two entry pathways targeted are independent. Using our modelling and analysis, we show how this synergy arises from effects both at the single cell level as well as at the cell population level. We show further that the synergy is evident in available \textit{in vitro} experiments with SARS-CoV-2 and SARS-CoV, providing evidence in strong support of our model predictions. Exploiting this synergy may help optimize combination therapies targeting TMPRSS2 and Cathepsin B/L for preventing SARS-CoV-2 entry.

\section*{Results}

\subsection*{Overview of the mathematical model}

We considered typical \textit{in vitro} experiments where a population of target cells is exposed in the presence or absence of protease inhibitors to virions expressing the SARS-CoV-2 spike protein S and the extent of infection is measured. We constructed a mathematical model of the ensuing dynamical processes, focusing on virus entry and the role of the protease inhibitors (Fig. 1). We assumed that cells could get infected following S protein activation by either TMPRSS2 or Cathepsin B/L. The efficiency of virus entry via the two pathways would depend on the expression levels of the respective proteases. We denoted as $S_t$ the susceptibility of a target cell expressing $n_t$ copies of TMPRSS2 to entry via the TMPRSS2 pathway. The susceptibility here was also the probability with which a virus particle could successfully enter the cell when TMPRSS2 was the sole
limiting factor. Analogously, $S_c$ was the susceptibility to entry via the Cathepsin B/L pathway of a cell expressing $n_c$ copies of Cathepsin B/L. The overall susceptibility, which we denoted $S_{ic}$, of a cell expressing $n_i$ and $n_c$ copies of the two proteases was then $S_{ic} = S_i + S_c - S_iS_c$, indicating the independence of the two pathways. We let $S_i$ and $S_c$ increase with $n_i$ and $n_c$, respectively, following distinct Hill functions. We assumed that drugs acted by suppressing the proteases in a dose-dependent manner. A TMPRSS2 inhibitor would thus block a fraction of the $n_i$ TMPRSS2 molecules, thereby reducing $S_i$. A Cathepsin B/L inhibitor would similarly lower $S_c$. The drugs thus acted independently in reducing $S_i$ and $S_c$, respectively. Furthermore, we assumed that a TMPRSS2 inhibitor left $S_c$ unaffected, so that entry could proceed uninhibited via the Cathepsin B/L pathway even when a TMPRSS2 inhibitor was present in excess. Similarly, a Cathepsin B/L inhibitor would let entry occur uninhibited via the TMPRSS2 pathway. Only when both the drugs were used could the cell be fully protected (Fig. 1A). We recognized finally that the cells in culture exhibited a distribution of the expression levels of TMPRSS2 and Cathepsin B/L and thus had a distribution of susceptibilities to infection via the two pathways (Fig. 1B). The effects of the drugs too, thus, exhibited cell-to-cell variability. With this description, we formulated dynamical equations to predict how the population of cells in culture would get infected as a function of time and how the drugs would suppress the infection (Methods). The model was solved using parameter values representative of viral infections in vitro. We also analysed the model and derived analytical expressions to estimate the extent of synergy between the drugs and to elucidate its origins (Methods).

The activity of TMPRSS2 and Cathepsin B/L inhibitors depends on the relative usage of the respective pathways

We considered cells expressing TMPRSS2 and Cathepsin B/L following independent, log-normal distributions (Fig. 2A, B). The susceptibilities, $S_i$ and $S_c$, of the cells to entry via the two pathways rose sigmoidally from zero at low expression levels to 1 at high expression levels of the respective proteases (Fig.
The expression levels of TMPRSS2 and Cathepsin B/L on cells followed a joint distribution which we assumed was the product of the individual distributions (Fig. 2C). The overall susceptibility, $S_c$, was small when both proteases were at low expression levels, rose with their expression levels, and reached 1 when either expression level was high (Fig. 2D). We employed this description of the protease expression levels and the corresponding susceptibilities in our model calculations. We recognize that the susceptibility could be altered by variations in the expression levels of other factors such as ACE2 or type I interferon-stimulated proteins, as seen in measurements$^{35,36}$ and evidenced by the substantial variability in the entry efficiency across different cell lines$^{15,37,38}$. Our focus here is on the relative entry efficiency through the two protease-activated pathways. The susceptibilities are thus relative to the maximum entry efficiency when factors other than TMPRSS2 and Cathepsin B/L are limiting.

Figure 1. Schematic of the mathematical model incorporating the two independent pathways of SARS-CoV-2 entry into target cells. (A) After the SARS-CoV-2 virion attaches to the host surface receptor ACE2, S protein activation occurs by the host proteases TMPRSS2 (transmembrane) or Cathepsin B/L (endosomal) thus yielding two independent entry pathways. Blocking both pathways is essential for preventing infection of the cell expressing both proteases. (B) TMPRSS2 and Cathepsin B/L expression levels vary across cells. The entry efficiency through a pathway increases and the efficacy of the inhibitor at a given concentration decreases with the expression level of the corresponding protease.
We examined first the influence of TMPRSS2 expression levels on virus entry. We considered scenarios where the mean TMPRSS2 level was low, medium, or high, corresponding to $S_1$ at the mean expression levels of 0.001, 0.5, and 0.95, respectively (Fig. 2E). Note that TMPRSS2 levels can vary substantially across tissues in the body, with the prostate, for instance, displaying significantly higher expression levels than the lung, trachea, or salivary glands. Different cell lines too express widely varying TMPRSS2 levels. For the distributions chosen, our model predicted that the total population of cells infected increased with TMPRSS2 expression, going from ~1640 cells/ml at low to ~5050 cells/ml infected at high mean TMPRSS2 expression level (Fig. 2F). The distribution of Cathepsin B/L expression (Fig. 2B) was unaltered in these calculations. The susceptibility through the Cathepsin B/L pathway at the mean Cathepsin B/L expression level was 0.029. Thus, the utilization of the TMPRSS2 pathway ($S_1/S_2$) was, on average, 0.03, 17.1, and 32.5 fold higher than the Cathepsin B/L pathway for the low, medium and high TMPRSS2 scenarios, respectively, indicating that as the TMPRSS2 level increased, it became the increasingly preferred entry pathway in our predictions.

We reasoned that as the preference for the TMPRSS2 pathway increased, it would increasingly compromise the activity of a Cathepsin B/L inhibitor. We examined this by performing the same calculations as above but now in the presence of a Cathepsin B/L inhibitor. Mimicking experiments, we calculated the fraction of cells ‘unaffected’ by the Cathepsin B/L inhibitor, which we denoted $f''(D_c)$ (Fig. 2G). Thus, $f''(D_c)=1$ would imply that the drug protected no cells from virus entry, whereas $f''(D_c)=0$ would imply that the drug protected all cells that would otherwise have been infected. Our model predicted that as the inhibitor level increased, $f''(D_c)$ decreased, indicating increased drug activity (Fig. 2G). $f''(D_c)$, however, did not reach 0 even at arbitrarily high drug concentrations, but instead plateaued to larger values depending on the mean TMPRSS2 expression level. When the latter expression level was low, $f''(D_c)$ plateaued at ~0.23, indicating strong protection, whereas when the expression level was high, $f''(D_c)$ was ~0.93, implying little protection. Thus, the ‘apparent’ efficacy of the Cathepsin B/L inhibitor was dependent on TMPRSS2 expression. The inhibitor was efficacious when the predominant pathway was via Cathepsin B/L, which here
happened when TMPRSS2 expression was low. When the latter expression was high, Cathepsin B/L was utilized negligibly; blocking it would thus have a minimal effect on virus entry, rendering the inhibitor poorly efficacious.

Figure 2. Predictions of SARS-CoV-2 entry into cells with heterogenous receptor expression levels. (A, B) The log-normal distribution of TMPRSS2 (A) and cathepsin B/L (B) across cells. Dependence of the susceptibility of infection through the TMPRSS2 pathway on the TMPRSS2 expression level, $n_r$, (A) and through the Cathepsin B/L pathway on the Cathepsin B/L expression level, $n_c$ (B). (C) The joint distribution of cells expressing both TMPRSS2 and Cathepsin B/L. (D) Dependence of susceptibility of infection on both TMPRSS2 and Cathepsin B/L expression levels. (E) The log-normal distribution of TMPRSS2 across cells
with low ($\overline{n_t} = 9.98$), medium ($\overline{n_t} = 11.7$) and high ($\overline{n_t} = 12.43$) mean TMPRSS2 expression levels. (F, G) Infected cells in the absence of inhibitors (F) and fraction of infection events uninhibited by different concentrations of a Cathepsin B/L inhibitor (G) at different mean TMPRSS2 expression levels and fixed mean Cathepsin B/L expression ($\overline{n_c} = 11.5$). (H) The log-normal distribution of Cathepsin B/L across cells with low ($\overline{n_c} = 10.67$), medium ($\overline{n_c} = 12.39$) and high ($\overline{n_c} = 13.12$) mean Cathepsin B/L expression levels. (I, J) Infected cells in the absence of inhibitors (I) and fraction of infection events uninhibited by different concentrations of TMPRSS2 inhibitor (J) at different mean TMPRSS2 expression levels and fixed mean Cathepsin B/L expression ($\overline{n_t} = 11.5$). In F, G, I, and J, the cumulative level of infection was computed at day 1 post infection, and the drug concentrations were normalised by the respective $\gamma$ values (Methods). Parameters: $\lambda = 0.77$ d$^{-1}$, $\mu = 0.22$ d$^{-1}$, $\delta = 0.53$ d$^{-1}$, $c = 10$ d$^{-1}$, $\beta = 1 \times 10^{-4}$ ml ffu$^{-1}$ d$^{-1}$, $n_{t0}^5 = 1.2 \times 10^5$ copies/cell, $n_{c0}^5 = 2.4 \times 10^5$ copies/cell, $\sigma_t = 1$, $\sigma_c = 1$, $h_t = 4$, and $h_c = 4$. In A-D, $\overline{n_t} = 11.5$ and $\overline{n_c} = 11.5$. Initial conditions: $T(0) = 1 \times 10^5$ cell ml$^{-1}$, $I(0) = 0$, and $V(0) = 1 \times 10^4$ ffu ml$^{-1}$.

In the same way, we predicted that the apparent activity of TMPRSS2 inhibitors would depend on the expression level of Cathepsin B/L. When we used low, medium and high mean expression levels of Cathepsin B/L in our calculations (Fig. 2H), with the TMPRSS2 expression level unaltered (Fig. 2A), we found that the extent of infection increased in the absence of drugs from ~2960 cells/ml at low to ~5390 cells/ml at high Cathepsin B/L expression (Fig. 2I). With a TMPRSS2 inhibitor, the extent of infection decreased, but the fraction unaffected, now denoted $f^u(D_f)$, plateaued to values that increased with the mean Cathepsin B/L expression level – $f^u(D_f) \sim 0.13$ at low and $f^u(D_f) \sim 0.88$ at high expression levels – indicating again that the apparent activity of the TMPRSS2 inhibitor decreased as the Cathepsin B/L pathway became increasingly preferred (Fig. 2J).

These predictions were consistent with several experiments showing that high expression levels of TMPRSS2 led to poor efficacies of Cathepsin B/L inhibitors. For instance, when 293T cells expressing human ACE2 but not TMPRSS2 were infected with SARS-CoV in the presence of 25 mM NH$_4$Cl, which prevents endosomal acidification and hence blocks Cathepsin B/L, the extent of infection reduced ~40-fold over that in the absence of NH$_4$Cl. When the same cells were engineered to express TMPRSS2, the reduction was just ~4-fold, marking a drastic loss of the apparent activity of NH$_4$Cl. Similar effects were observed with a host of other endosomal pathway inhibitors including E-64d, MDL28170, EST, and bafilomycin. Importantly, recent experiments observed this effect with SARS-CoV-2. When 293 T cells expressing ACE2
were infected with SARS-CoV-2 pseudoparticles, treatment with E-64d reduced the infection by 90%, whereas when the cells additionally expressed TMPRSS2, the effect of E-64d nearly vanished.\(^\text{15}\)

These predictions were also consistent with experiments showing high activity of TMPRSS2 inhibitors when the expression level of TMPRSS2 was high.\(^\text{15,19}\) For instance, with HeLa cells expressing ACE2, the effect of camostat mesylate was negligible in inhibiting SARS-CoV infection compared to its effect on the same cells engineered to overexpress TMPRSS2.\(^\text{19}\) Importantly, with the latter cells too, camostat mesylate prevented only \(~65\)% of the cells from getting infected even at the highest doses, consistent with the plateau predicted by our model (Fig. 2G, J). The implication was that the cells utilized the Cathepsin B/L pathway \(~35\)% of the time on average, which the TMPRSS2 inhibitor could not block. In the same way, with Vero cells, which hardly express TMPRSS2, camostat mesylate had hardly any effect in reducing SARS-CoV-2 infection, whereas E-64d, which blocked Cathepsin B/L reduced nearly 100% of the infections.\(^\text{15}\) At the same time, in Vero cells engineered to overexpress TMPRSS2, with the same dosages of the drugs, E-64d could only block \(~25\)% of the infections, whereas camostat mesylate blocked nearly \(~65\)% of the infections, indicative again of the relative utilization of the pathways in these cell lines. Similar results were observed also with SARS-CoV infection.\(^\text{15}\)

These findings suggest caution when interpreting assays measuring the efficacies of drugs targeting host proteases. A drug may block its target protease potently but may still appear poorly efficacious at preventing viral entry because of the activity of the other protease. More importantly, the findings suggest that with cells expressing significant levels of both TMPRSS2 and Cathepsin B/L, the use of either a TMPRSS2 inhibitor or a Cathepsin B/L inhibitor alone would be insufficient to block entry completely. The inhibitors would have to be used together to completely block entry. We therefore examined next our model predictions when the inhibitors were used together.

The combined use of TMPRSS2 and Cathepsin B/L inhibitors can fully block entry

To elucidate the effect of combination therapy, we picked the distributions of TMPRSS2 and Cathepsin B/L expression levels such that both the inhibitors displayed significant effects independently. Thus, we let the
effect of the Cathepsin B/L inhibitor plateau at \( f^u(D_C) \approx 0.78 \) and that of the TMPRSS2 inhibitor plateau at \( f_u(D_T) \approx 0.39 \) when used alone (Fig. 3A). As expected, the drugs independently could not block infections completely. When the two were combined, our model predicted 100% block of entry; the fraction unaffected, now denoted \( f^u(D_T, D_C) \), plateaued at 0 (Fig. 3A).

This prediction was consistent, again, with several \textit{in vitro} studies showing that full blockade of entry required the combined use of TMPRSS2 and Cathepsin B/L inhibitors.\textsuperscript{15,19} With HeLa cells expressing ACE2 and TMPRSS2, EST alone blocked \(~34\%) of SARS-CoV infection, camostat mesylate blocked \(~58\%\), and the two together blocked \(~100\%) of the infections.\textsuperscript{19} In Vero cells expressing TMPRSS2, the corresponding numbers were \(~20\%) for E-64d, \(~47\%) for camostat mesylate, and \(~98\%) for both with SARS-CoV infection.\textsuperscript{15} Importantly, the latter numbers were 21\%, 57\%, and \(~92\%) respectively, for SARS-CoV-2 infection.\textsuperscript{15}

\textbf{TMPRSS2 and Cathepsin B/L inhibitors synergize in blocking virus entry}

The above predictions indicated strong synergy between drugs blocking the two entry pathways (Fig. 3A). Because the pathways are independent, the combined effect of the drugs is expected to follow Bliss independence, where the fraction of events unaffected by the combination is the product of the fractions unaffected when the drugs are used alone.\textsuperscript{31,34,41} In other words, Bliss independence would imply

\[
 f^u_{\text{Bliss}}(D_T, D_C) = f^u(D_T) \times f^u(D_C) .
\]

If \( f^u(D_T, D_C) \) is smaller than \( f^u_{\text{Bliss}}(D_T, D_C) \), the drugs exhibit synergy; \textit{i.e.}, they block more infections together than expected from their individual effects put together. With the above parameter values, where individual effects plateaued at \( f_u(D_T) \approx 0.78 \) and \( f^u(D_C) \approx 0.39 \), the expected effect following Bliss independence would be \( f^u_{\text{Bliss}}(D_T, D_C) \approx 0.3 \). The fraction predicted by our model, on the other hand, was \( f^u(D_T, D_C) = 0 \), implying strong synergy (Fig. 3A).

The synergy can be visualized in two ways\textsuperscript{31}: horizontal and vertical synergy (Fig. 3A). Horizontal synergy refers to the decrease in drug levels from that assuming independent action that is required to produce the same effect as that achieved by Bliss independence. Vertical synergy refers to the gain in efficacy at fixed
drug levels over that assuming Bliss independence. The two are equivalent ways of quantifying synergy. The latter has been termed Bliss synergy and is more widely used. Here, we employed the latter measure throughout, and denoted it as \( \beta_{\text{Bliss}} = f''_{\text{Bliss}}(D_T, D_C) - f''(D_T, D_C) \). As the extent of synergy increases, \( \beta_{\text{Bliss}} \) increases. Note that \( 0 \leq \beta_{\text{Bliss}} \leq 1 \).

With the above parameters, we varied drug levels over wide ranges and calculated for each combination of drug levels, the efficacy (in terms of the fraction unaffected) when the drugs were used alone as well as in combination and obtained the expected efficacy from Bliss independence (Fig. 3B), the predicted efficacy of combination therapy (Fig. 3C) and the extent of Bliss synergy (Fig. 3D). The combined efficacy expected assuming Bliss independence was small \( (f''_{\text{Bliss}}(D_T, D_C) \sim 1) \) when the drug levels were small and rose to the maximum estimated above \( (f''(D_T, D_C) \sim 0.3) \) at saturating drug levels (Fig. 3B). In striking contrast, the predicted efficacy of the combination was much higher at saturating drug levels \( (f''(D_T, D_C) \sim 0) \) (Fig. 3C). Correspondingly, \( \beta_{\text{Bliss}} \sim 0.3 \), which quantified the maximum extent of synergy (Fig. 3D). When one of the drugs was used at low concentrations, synergy was low, as expected from the dominance of the activity of the other drug. When both the drugs were used at significant levels, the extent of synergy rose, and eventually attained the maximum above at saturating drug levels (Fig. 3D).

These predictions of synergy were unexpected because the two drugs targeted independent pathways and were thus supposed to obey Bliss independence. We therefore examined next the possible origins of the synergy in our model predictions.

**Origin of synergy at the single cell level**

Synergy could arise from the effects of the drugs at the single cell level or at the cell population level. We sought to examine first whether synergy could arise in our calculations from effects at the single cell level. For this, we considered cells that had the same TMPRSS2 and Cathepsin B/L levels, eliminating heterogeneity in the cell population. Any synergy would then have to arise from effects at the single cell level. We performed our calculations above with this homogeneous cell population.
Figure 3. Predictions of the effect of combination treatment targeting both TMPRSS2 and Cathepsin B/L pathways. (A) The fraction of infection events unaffected by Cathepsin B/L inhibitor, TMPRSS2 inhibitor or both for different drug concentrations. The extent of predicted horizontal and vertical or Bliss synergy (see text) are marked. (B, C) The expected effect of the combination from Bliss independence (B) and predicted combination effect (C) over varying drug concentrations. (D) The predicted Bliss synergy over a range of drug concentrations. Parameters: $\lambda = 0.77$ d$^{-1}$, $\mu = 0.22$ d$^{-1}$, $\delta = 0.53$ d$^{-1}$, $c = 10$ d$^{-1}$, $\beta = 1 \times 10^{-4}$ ml ffu$^{-1}$ d$^{-1}$, $n_{i_0} = 1.2 \times 10^5$ copies/cell, $n_{c_0} = 2.4 \times 10^5$ copies/cell, $\bar{n}_i = 11.5$, $\bar{n}_c = 11.5$, $\sigma_i = 1$, $\sigma_c = 1$, $\theta_i = 4$, and $\theta_c = 4$. Initial conditions: $T(0) = 1 \times 10^5$ cell ml$^{-1}$, $I(0) = 0$, and $V(0) = 1 \times 10^4$ ffu ml$^{-1}$.

Mimicking the results above (Figs. 2 and 3), we found from our predictions that for fixed Cathepsin B/L levels, as the expression level of TMPRSS2 increased from low to high, the number of cells infected increased from $\sim 870$/ml to $\sim 6240$/ml in the absence of drugs (Fig. 4A). With a Cathepsin B/L inhibitor, $f''(D_c)$ decreased and reached a plateau (Fig. 4B). The plateau was lower, indicating greater drug efficacy, as the TMPRSS2 level decreased, consistent with the observations above of the drug efficacy increasing as the relative usage of the corresponding pathway increases. Analogous predictions resulted upon increasing
Cathepsin B/L expression levels at a fixed TMPRSS2 level (Fig. 4C) and in the presence of increasing concentrations of a TMPRSS2 inhibitor (Fig. 4D).

We now performed calculations with both the drugs present simultaneously. We chose protease expression levels such that the individual drug effects plateaued at $f_a(D_T) \approx 0.32$ and $f_a(D_C) \approx 0.79$, respectively (Fig. 4E). In the absence of synergy, the expected combined effect would plateau at $f_{Bliss}(D_T, D_C) \approx 0.26$. Our model predicted instead that the combined effect plateaued at $f_a(D_T, D_C) \approx 0$, indicating strong synergy at the single cell level (Fig. 4E). Specifically, here, the Bliss synergy, denoted $\beta_{Bliss}^{cell}$ to emphasize its origin, was $\beta_{Bliss}^{cell} \approx 0.26$. When we repeated the above calculations over a wide range of drug concentrations, we found that the expected effect of the combination, $f_{Bliss}(D_T, D_C)$, did not fall below $\approx 0.26$ (Fig. 4F), whereas the predicted effect, $f_a(D_T, D_C)$, reached zero (Fig. 4G), and the corresponding synergy was close to $\beta_{Bliss}^{cell} \approx 0.26$ at high concentrations of both the drugs, reiterating the existence of synergy at the single cell level (Fig. 4H).

The above calculations were for fixed TMPRSS2 and Cathepsin B/L levels. To ascertain the robustness of the results, we repeated the calculations with different levels of either TMPRSS2 (Fig. 4I) or Cathepsin B/L (Fig. 4J), while keeping all the other parameters fixed. We found that the synergy had a non-monotonic dependence on the expression levels. As the TMPRSS2 level increased, for instance, $\beta_{Bliss}^{cell}$ rose from zero, attained a peak that was dependent on the drug level, and then declined again to zero (Fig. 4I). We explain this effect by considering again the relative preferences for the two entry pathways. When the TMPRSS2 level was low, its contribution to entry was low, so that only Cathepsin B/L inhibitors would display significant efficacy. The effect of the TMPRSS2 inhibitor was imperceptible. No synergy could arise. Conversely, when the TMPRSS2 levels were high, Cathepsin B/L inhibitors had hardly any effect. Synergy was again not possible. At intermediate expression levels, synergy could occur and was maximized. With increasing drug levels, this effect was amplified because each drug blocked their respective pathways more effectively, leading to
increased overall synergy at intermediate expression levels. Analogous effects were evident with varying Cathepsin B/L expression levels (Fig. 4J).

Figure 4. Predictions of SARS-CoV-2 entry into cells with homogenous receptor expression levels. (A, B) Cells infected in the absence of inhibitors (A) and fraction of infection events uninhibited by different concentrations of Cathepsin B/L inhibitor (B) in a population of cells expressing low \( n_r = 20,000 \) copies/cell, medium \( n_r = 100,000 \) copies/cell) and high \( n_r = 500,000 \) copies/cell) TMPRSS2 levels and fixed \( n_c = 150,000 \) copies/cell) cathepsin B/L level. (C, D) Cells infected in the absence of inhibitors (C) and fraction of infection events uninhibited by different concentrations of TMPRSS2 inhibitor (D) in a population of cells expressing low \( n_c = 20,000 \) copies/cell), medium \( n_c = 170,000 \) copies/cell) and high \( n_c = 500,000 \) copies/cell).
copies/cell) cathepsin B/L levels and fixed \((n_i = 100,000 \text{ copies/cell})\) TMRPSS2 level. (E) The fraction of infection events unaffected by cathepsin B/L inhibitor, TMRPSS2 inhibitor or both for different drug concentrations in cell population with \(n_i = 100,000 \text{ copies/cell}\) and \(n_c = 150,000 \text{ copies/cell}\). (F, G) The expected combination effect from Bliss independence \((F)\) and predicted combination effect \((G)\) over a varying levels of drug concentrations. (H) The predicted Bliss synergy over a range of drug concentrations. (I, J) The predicted Bliss synergy for varying TMRPSS2 expression and fixed \((n_c = 150,000 \text{ copies/cell})\) cathepsin B/L expression and for varying cathepsin B/L expression and fixed \((n_i = 100,000 \text{ copies/cell})\) TMRPSS2 expression at two different drug concentrations. The drug concentrations were normalised by the respective \(\gamma\) values. Parameters: \(\lambda = 0.77 \text{ d}^{-1}\), \(\mu = 0.22 \text{ d}^{-1}\), \(\delta = 0.53 \text{ d}^{-1}\), \(c = 10 \text{ d}^{-1}\), \(\beta = 1 \times 10^{-4} \text{ ml ffu}^{-1} \text{ d}^{-1}\), \(n^{50}_i = 1.2 \times 10^5 \text{ copies/cell}\), \(n^{50}_c = 2.4 \times 10^5 \text{ copies/cell}\), \(h_i = 4\), and \(h_c = 4\). Initial conditions: \(T(0) = 1 \times 10^5 \text{ cell ml}^{-1}\), \(I(0) = 0\), and \(V(0) = 1 \times 10^4 \text{ ffu ml}^{-1}\).

To understand the above observations more deeply, we considered a single round of infection, typical of pseudotyped virus assays\(^{15,19}\), for which we could construct analytical expressions using our model (Methods). Because we assumed a homogeneous cell population in our calculations above, we could focus on a single cell. We considered a cell with susceptibilities through the two pathways denoted \(S_i\) and \(S_c\), respectively. It followed that the probability of it getting infected in the absence of drugs in a single round of infection would be \(S_{\text{uc}} = S_i + S_c - S_iS_c\). With the drugs, we let the susceptibilities be reduced to \(S_i(D_T) \leq S_i\) and \(S_c(D_C) \leq S_c\), respectively. With the TMRPSS2 inhibitor alone, the probability of infection would be \(S_{\text{u}i}(D_T) = S_i(D_T) + S_c(D_T)S_i\), whereas it would be \(S_{\text{uc}}(D_C) = S_i + S_c(D_C) - S_iS_c(D_C)\) with a Cathepsin B/L inhibitor alone. With the drugs used together, the probability would become \(S_{\text{uc}}(D_T, D_C) = S_i(D_T) + S_c(D_C) - S_i(D_T)S_c(D_C)\). Because the cells were assumed to be identical, the probabilities, when the cell numbers are large, would correspond to the fractions of cells infected in the respective scenarios. Thus, the fraction unaffected by the drugs was given by the ratios, \(f^u(D_T) = S_{\text{uc}}(D_T) / S_{\text{uc}}\), \(f^u(D_C) = S_{\text{uc}}(D_C) / S_{\text{uc}}\) and \(f^u(D_T, D_C) = S_{\text{uc}}(D_T, D_C) / S_{\text{uc}}\). Using the expressions above and simplifying, the Bliss synergy was, \(\rho_{\text{Bliss}} = f^u(D_T)f^u(D_C) - f^u(D_T, D_C) = (S_i - S_i(D_T))(S_c - S_c(D_C)) / (S_i + S_c - S_iS_c)^2\). This expression explained the trends predicted above. When the TMRPSS2 expression level was low, for instance, \(S_i\) was low and so was \(S_i(D_T)\), implying a small difference between the two, and hence weak synergy. The
same argument applied to Cathepsin B/L expression and inhibition. When both the proteases were expressed in significant amounts, the differences $S_i - S_i(D_T)$ and $S_c - S_c(D_c)$ became significant and sizeable synergy resulted. With increasing drug levels, the differences were amplified, leading to greater synergy. At very high expression levels of TMPRSS2, $S_i$ approached unity and so did $S_i(D_T)$ because the drug could not block a large enough fraction of the protease molecules to lower susceptibility. The difference between $S_i$ and $S_i(D_T)$ thus diminished, lowering synergy and explaining the non-monotonic trend in synergy observed with drug levels (Figs. 4I and 4J).

Overall, these predictions indicated that strong synergy could arise from the effects of the two drugs at the single cell level. We examined next whether synergy could also arise from effects at the cell population level.

**Origin of synergy at the cell population level**

Synergy at the cell population level can arise when heterogeneity exists in the response of cells to the drugs involved. Here, such heterogeneity could arise from the distributions of the expression levels of the two proteases across cells. To illustrate the concept, we considered the extreme scenario where cells in culture either expressed TMPRSS2 or Cathepsin B/L but not both. We were able to derive analytical expressions to demonstrate synergy from the cell population level in this scenario. We let the fraction of cells expressing TMPRSS2 be $\phi_i$ and the fraction expressing Cathepsin B/L be $\phi_c = 1 - \phi_i$. Further, we let the expression levels of TMPRSS2 in the first subpopulation and Cathepsin B/L in the second be fixed, so that no heterogeneity existed within the subpopulations. The first subpopulation was thus susceptible to entry only via the TMPRSS2 pathway and thus could respond to TMPRSS2 inhibitors alone. We let the susceptibilities of this subpopulation without and with a TMPRSS2 inhibitor be $S_i$ and $S_i(D_T)$, respectively. Similarly, the second subpopulation was susceptible to entry via the Cathepsin B/L pathway alone and responded to Cathepsin B/L but not TMPRSS2 inhibitors. We set the corresponding susceptibilities to $S_c$ and $S_c(D_c)$, respectively. Neither subpopulation admitted synergy at the single cell level, for each could be affected by only
one of the drugs. The cell population thus exhibited no synergy arising from effects at the single cell level. We now considered subjecting the cell population comprised of the two subpopulations to different treatments.

In the absence of treatment, the total fraction of cells infected would be $f^{\text{inf}} = \phi_i S_i + \phi_c S_c$. With a TMPRSS2 inhibitor alone, the fraction would become $f^{\text{inf}}(D_T) = \phi_i S_i (D_T) + \phi_c S_c$, whereas it would be $f^{\text{inf}}(D_C) = \phi_i S_i + \phi_c S_c(D_C)$ with a Cathepsin B/L inhibitor alone. In the presence of both drugs, the fraction infected would become $f^{\text{inf}}(D_T, D_C) = \phi_i S_i(D_T) + \phi_c S_c(D_C)$. The fractions unaffected by the drugs in the different scenarios would be given by the ratios, $f^{u}(D_T) = f^{\text{inf}}(D_T)/f^{\text{inf}}$, $f^{u}(D_C) = f^{\text{inf}}(D_C)/f^{\text{inf}}$ and $f^{u}(D_T, D_C) = f^{\text{inf}}(D_T, D_C)/f^{\text{inf}}$. Using these expressions, we derived an expression for the Bliss synergy, now denoted $\beta_{\text{Bliss}}^{\text{popn}}$ to emphasize its origin in the cell population level. We obtained

$$\beta_{\text{Bliss}}^{\text{popn}} = f^{u}(D_T)f^{u}(D_C) - f^{u}(D_T, D_C) = \phi_i \phi_c(S_i - S_c(D_T))(S_c - S_c(D_C))/(\phi_i S_i + \phi_c S_c)^2.$$  

It followed that this synergy would vanish in the absence of cellular heterogeneity; i.e., when $\phi_i = 0$ or $\phi_i = 1$, implying the presence of just one of the two subpopulations. The synergy was maximum when the two subpopulations were fully susceptible ($S_i = S_c = 1$), fully drug responsive ($S_i(D_T) = S_c(D_C) = 0$) and equally populous ($\phi_i = \phi_c = 1/2$). The corresponding maximum synergy was $\beta_{\text{Bliss}}^{\text{popn}} = 1/4$.

This limiting, two subpopulation scenario served to elucidate the origin of synergy at the cell population level. Because of the heterogeneity in the utilization of the entry pathways, neither drug could block the infection of both the subpopulations. Both drugs together, however, could protect the entire cell population.

To assess the extent of synergy arising from the cell population level in a setting representative of the cell cultures used in experiments, we generalized the arguments above to the scenario we considered in our calculations in Figs. 2 and 3 (Methods). We found that when the total synergy reached a plateau at $\sim 0.3$, the cell population level synergy was $\sim 0.13$, amounting to nearly 43% of the total synergy (Fig. 5A). By varying drug levels over wide ranges, we found that the synergy was low at low drug levels and increased monotonically as drug levels rose (Fig. 5B). The single cell level synergy displayed similar trends, leading to the overall synergy also rising monotonically with drug levels and eventually saturating at $\sim 0.3$ (Fig. 5C).
recognized from our analysis of the limiting scenarios above that \( \beta_{\text{Bliss}}^{\text{popn}} \) is bounded above by \( \frac{1}{4} \), whereas \( \beta_{\text{Bliss}}^{\text{cell}} \) can rise to 1. Thus, depending on the overall synergy, the contribution from the cell population level may vary. Whereas it was \(~43\%\) above, it could be much lower if \( \beta_{\text{Bliss}}^{\text{popn}} \) were smaller and/or \( \beta_{\text{Bliss}}^{\text{cell}} \) were larger. To illustrate this, we performed calculations with parameter settings that yielded a much higher overall synergy as well as a lower cell population level synergy. Here, the overall synergy saturated at 0.69, the cell population synergy saturated at 0.03, resulting in a far smaller contribution from the latter of 4.4\% (Fig. 5D, E, F). Finally, we note that the cell population level synergy too displayed non-monotonic dependencies on the expression levels of the proteases (Figs. 5G and H). These trends mimic those with the single cell level synergy (Figs. 4I and J) and are readily understood, following the arguments above, as arising from the magnitudes of the differences \( S_i - S_i(D_r) \) and \( S_c - S_c(D_c) \).

In summary, it followed from these model predictions that contributions could arise to the synergy from effects of the drugs at the single cell as well as cell population levels. We examined next whether the synergy we predicted was evident in available \textit{in vitro} data on SARS-CoV-2 and SARS-CoV infection.

**Evidence of synergy between TMPRSS2 and Cathepsin B/L inhibitors \textit{in vitro}**

We considered data from 4 independent experiments, 1 with SARS-CoV-2 and 3 with SARS-CoV, performed across 2 different cell lines, using camostat mesylate as the TMPRSS2 inhibitor and 3 different drugs as Cathepsin B/L inhibitors, in two independent studies that have examined the combined effect of blocking TMPRSS2 and Cathepsin B/L\textsuperscript{15,19}. The studies exposed cells to pseudo-typed viruses in the absence of drugs or in the presence of either or both drugs and reported the fraction of infection events unaffected by the drugs, using which we estimated the extent of Bliss synergy, \( \beta_{\text{Bliss}} \), between the drugs (Methods). We found that all the experiments displayed synergy between TMPRSS2 and Cathepsin B/L inhibitors, validating our model predictions (Table 1).
Figure 5. Predictions of population level synergy. (A-F) The predicted population level and overall synergy over a range of drug concentrations (see text for details). (G, H) The predicted overall and population level synergy for varying mean TMPRSS2 expression and fixed mean ($\overline{n}_c = 11.5$) cathepsin B/L expression (G) and for varying mean cathepsin B/L expression and fixed mean ($\overline{n}_t = 11.5$) TMPRSS2 expression at two different drug concentrations (H). The drug concentrations were normalised by the respective $\gamma$ values. In A-C and G-H, $n_{t_0}^{50} = 1.2 \times 10^5$ copies/cell and $n_{c_0}^{50} = 2.4 \times 10^5$ copies/cell. In D-F, $n_{t_0}^{50} = 4 \times 10^4$ copies/cell, $n_{c_0}^{50} = 4 \times 10^4$ copies/cell. Parameters: $\lambda = 0.77 \text{ d}^{-1}$, $\mu = 0.22 \text{ d}^{-1}$, $\delta = 0.53 \text{ d}^{-1}$, $c = 10 \text{ d}^{-1}$, $\beta = 1 \times 10^{-4}$ ml ffu$^{-1}$ d$^{-1}$, $\overline{n}_t = 11.5$, $\overline{n}_c = 11.5$, $\sigma_t = 1$, $\sigma_c = 1$, $h_t = 4$, $h_c = 4$. Initial conditions: $T(0) = 1 \times 10^5$ cell ml$^{-1}$, $I(0) = 0$, and $V(0) = 1 \times 10^4$ ffu ml$^{-1}$. 


With SARS-CoV-2, we estimated $\beta_{\text{Bliss}} \approx 0.26$ between camostat mesylate and E-64d in Vero cells expressing TMPRSS2, suggesting that the combination protects an additional 26% of target cells from being infected relative to the additive effects of the two drugs at the same level of exposure. Strong synergy was also seen with SARS-CoV, with $\beta_{\text{Bliss}} \approx 0.4$ under the same conditions. The synergy extended to other drug combinations and cell lines. With camostat mesylate and EST, the latter a broad inhibitor of cysteine proteases including Cathepsin B/L, $\beta_{\text{Bliss}} \approx 0.22$ with SARS-CoV infection in HeLa cells expressing ACE2 and TMPRSS2. With camostat mesylate and bafilomycin, which prevents endosomal acidification and thus blocks Cathepsin B/L function, the corresponding estimate was $\beta_{\text{Bliss}} \approx 0.17$.

| Infection | Cell line      | Drug combination             | $f^\nu(D_T)$ | $f^\nu(D_C)$ | $f^\nu(D_T,D_C)$ | $\beta_{\text{Bliss}}$ | Ref |
|-----------|----------------|------------------------------|--------------|--------------|------------------|------------------------|-----|
| SARS-2-S  | Vero-TMPRSS2   | Camostat ($D_T$) + E-64d ($D_C$) | 0.43         | 0.79         | 0.34             | 0.08                   | 0.26 | 15 
| SARS-S    | Vero-TMPRSS2   | Camostat ($D_T$) + E-64d ($D_C$) | 0.53         | 0.8          | 0.42             | 0.02                   | 0.4  | 15 
| SARS-S    | HeLa-ACE2-TMPRSS2 | Camostat ($D_T$) + EST ($D_C$) | 0.42         | 0.66         | 0.28             | 0.06                   | 0.22 | 19 |
| SARS-S    | HeLa-ACE2-TMPRSS2 | Camostat ($D_T$) + Bafilomycin ($D_C$) | 0.42         | 0.6          | 0.25             | 0.08                   | 0.17 | 19 |

**Table 1: Experimental evidence of Bliss synergy.** We computed the extent of synergy from reported data on the effects of the individual drugs and the combination. The difference between the expected effect of the combination in terms of the fraction unaffected ($f^\nu$) based on Bliss independence and the effect observed yielded the extent of synergy. Infection was by viruses pseudotyped with either SARS-CoV-2 S or SARS-CoV S protein.

![Bliss synergy scale](image)

$\beta_{\text{Bliss}}$

| 0.1 | 0.2 | 0.3 | 0.4 |
This evidence of the strong presence of synergy between TMPRSS2 and Cathepsin B/L inhibitors validated our model predictions. The synergy could be exploited in defining optimal drug combinations targeting host proteases required for SARS-CoV-2 entry, as has been suggested in other contexts\textsuperscript{31,34,42,43,45}.

Discussion

The unprecedented scale of the COVID-19 pandemic has spurred urgent efforts to develop drugs and vaccines against SARS-CoV-2. Targeting host proteases required for SARS-CoV-2 entry into target cells appears a promising option and is under clinical investigation. Here, we identified unexpected synergy between drugs that target the serine protease TMPRSS2 and the cysteine proteases Cathepsin B/L, which offer alternative and independent pathways for SARS-CoV-2 entry. Using mathematical modelling of SARS-CoV-2 entry via the two pathways, we showed that the synergy is novel and arises from effects both at the single cell level and the cell population level. We found analysing several reported \textit{in vitro} experimental observations that the synergy was evident not only with SARS-CoV-2 but also with the closely related and much more extensively studied SARS-CoV infection, which uses the same entry pathways, and was observed across drugs and cell lines, suggesting that the synergy is a robust phenomenon. A drug targeting TMPRSS2, namely, camostat mesylate, as well as drugs targeting the Cathepsin B/L pathway, such as hydroxychloroquine, are in independent clinical trials for SARS-CoV-2 treatment.\textsuperscript{3,11} Exploiting the synergy may maximize the impact of such drugs and improve the chances of curing SARS-CoV-2 infection.

Synergy can be of particular importance in the use of repurposed drugs. Because repurposed drugs are designed primarily for other, typically unrelated targets, they are expected to display suboptimal efficacies against their newly intended targets. Indeed, a recent analysis of SARS-CoV-2 dynamics in 13 patients treated with different repurposed drugs found that the drug efficacies were in the range of 20-70\% and were far below the minimum of 80\% that would be required for effective treatment after the onset of symptoms.\textsuperscript{49} Increasing drug levels to achieve desired efficacies is likely to be limited by toxicities. Synergy here could be particularly
helpful for it would allow the realization of the desired efficacies at drug concentrations below what would be required had the drugs acted independently. Thus, the synergy between TMPRSS2 inhibitors and Cathepsin B/L inhibitors we unravelled may improve their deployability against SARS-CoV-2.

The synergy we elucidated is unconventional and arises because two independent entry pathways are accessible to SARS-CoV-2. Classically, synergy is thought to arise between drugs when one of the drugs potentiates the other through interactions between their targets or downstream pathways.\(^{31,32,34,41,43}\) No such interaction is evident here. The synergy here arises because blocking both pathways is necessary for preventing entry and can only be achieved when both the drugs are used. Such synergy has been recognized earlier in the context of biochemical pathways where the same pathway can be triggered by two upstream stimuli independently.\(^{31}\) Drugs that block the two stimuli, seemingly independent targets, display synergy, in a manner similar to what we have elucidated here. In addition, synergy arises in our case from the variations in the expression levels of the two proteases across cells. Because of the variations, some cells predominantly use one entry pathway and others the other pathway. Thus, protecting the entire cell population is achieved efficaciously when both the drugs are used. Such cell population level synergy has been argued to explain the observed synergy between drugs targeting hepatitis C virus entry and those targeting other steps of the hepatitis C virus lifecycle including type I interferons.\(^{46,50}\) The overall synergy seen here is thus a convolution of the synergy at the single cell level as well as that at the cell population level. To our knowledge, such multi-level synergy between drugs has not been recognized earlier.

The multi-level synergy implies that the use of the drugs in combination may address redundancies at multiple levels that sustain the infection. The redundancy at the level of protease usage for infecting individual cells is well recognized.\(^{15,19}\) The combination would block both TMPRSS2 and Cathepsin B/L, addressing the redundancy and thereby protecting cells. Redundancy may also arise at the cell population level. The cell tropism of SARS-CoV-2 in humans is only beginning to be identified and is speculated to be larger than SARS-CoV.\(^{36}\) The expression level of TMPRSS2 varies across tissues in humans and mice.\(^{35,36,39}\) It is possible thus that in some tissues, with high TMPRSS2 expression, SARS-CoV-2 predominantly use TMPRSS2 for entry, whereas in others, with low TMPRSS2 expression, it uses Cathepsin B/L. Protecting all the tissues is thus only
possible with the combination. If the extent of these redundancies in vivo, which remains to be ascertained, is high, the extent of synergy and hence the efficacy of the combination would be high.

Our model predicted that the extent of synergy varied non-monotonically with receptor expression levels. With a low expression level of a particular receptor, the drug targeting it would have little role because entry would primarily occur through the alternative pathway. With a high expression level, the drug may not be able to block a significant fraction of the receptors and preclude entry. In both cases, thus, little synergy was possible. Knowledge of the expression levels of TMPRSS2 and Cathepsin B/L in the cells targeted by SARS-CoV-2 would help define drug levels that would block the pathways suitably so that maximal synergy can be realized. Given a desired efficacy of the combination, arising from considerations, for instance, of achieving a desired decline in viral load\textsuperscript{49}, optimal drug dosages can be estimated that would maximize synergy while ensuring efficacy, as has been demonstrated in other settings\textsuperscript{31,34,43,45,51}. Our model provides a formalism that can be applied to predict such optimal dosages with combinations of TMPRSS2 and Cathepsin B/L inhibitors. The optimization would require knowledge of the distributions of the proteases across cells and across the relevant cell and tissue types targeted by SARS-CoV-2, which is just beginning to be acquired\textsuperscript{35,36}.

That the synergy here is novel is made further evident by it not being restricted by the trade-off with efficacy, which has been shown recently to constrain a variety of synergistic drug combinations, including those used in HIV, hepatitis C and cancer treatments\textsuperscript{43,45}. The trade-off implies that as the efficacy of a combination increases beyond a point, the synergy decreases, thereby limiting the synergy that can be realized given a desired efficacy. This trade-off originates from the classical drug interactions underlying synergy, where, for instance, one drug potentiates the other in blocking different steps of the same pathway.\textsuperscript{43} If one of the drugs is used at such high efficacies that the pathway is nearly completely blocked, then little room is left for the other drug to act and no synergy is possible. This trade-off is not expected to constrain the combination of TMPRSS2 and Cathepsin B/L inhibitors because the two target independent pathways and not different steps in the same pathway. Indeed, our model predictions showed that the synergy would increase with efficacy, implying a gain in synergy with increasing drug concentrations. The synergy may be compromised, however,
and become constrained by the trade-off if one of the drugs were to exhibit activity against both the pathways, as has been suggested with the drug TLCK\textsuperscript{19}.

The elucidation of this novel synergy between TMPRSS2 inhibitors and Cathepsin B/L inhibitors required advances in mathematical modelling and analysis. Mathematical models have been developed earlier to describe receptor-dependent virus entry, with a focus on defining the entry requirements of viruses and quantifying targets of entry inhibitors and vaccines\textsuperscript{46,52–61}. The models, however, have focussed typically on a single entry pathway, although multiple proteins may have been involved in mediating entry through the pathway. For instance, models of HIV-1 entry, which requires the binding of the HIV-1 envelope gp120 to the cell surface receptor CD4 and the chemokine receptor CCR5 (or CXCR4), have focused on estimating the number of gp120 trimers that must engage with CD4 and CCR5 for successful entry\textsuperscript{53–55,58}. Similarly, models of hepatitis C virus entry have focused on the number of viral envelope proteins E2 that must bind with the cell surface receptors CD81 for successful entry\textsuperscript{52,56} (In one study, the presence of another cell surface molecule SR-BI has been shown to aid hepatitis C virus entry, but entry without CD81 binding has not been demonstrated\textsuperscript{56}). Accordingly, the models rarely admitted multiple entry pathways. Our model considered two independent entry pathways, allowing the elucidation of the synergy achieved by blocking them both. Furthermore, by using suitably designed limiting or extreme scenarios, our analysis brought out conceptually the origins of the synergy both at the single cell and the cell population levels. The resulting findings are likely to apply beyond SARS-CoV-2 because the entry of several other viruses, including other betacoronaviruses such as SARS-CoV, MERS-CoV, and influenza viruses\textsuperscript{62}, occurs similarly, via two independent pathways.

We expect our study to prompt investigations into the usage of the two pathways by SARS-CoV-2 \textit{in vivo}, where the synergy we elucidated remains to be established. Studies on SARS-CoV infection of mice have remained inconclusive on this synergy. In some studies, synergy between camostat mesylate and drugs targeting Cathepsin B/L was not observed in mice, although it was evident \textit{in vitro}\textsuperscript{63,64}. Studies with the coronavirus HCoV229E, among those causing common cold, attributed this difference between \textit{in vitro} and \textit{in vivo} settings to mutations associated with the laboratory adaptation of the virus to cell lines\textsuperscript{55,66}. The mutations have been argued to allow Cathepsin B/L usage \textit{in vitro}, while the \textit{in vivo} strains predominantly use TMPRSS2.
Yet, successful SARS-CoV infection of TMPRSS2 knockout mice was observed, demonstrating the ability of SARS-CoV and suggesting the ability of SARS-CoV-2 to use Cathepsin B/L \textit{in vivo}.\textsuperscript{63} Besides, the proposed laboratory adaptation appears unlikely to have happened with SARS-CoV-2 because the virus isolated from infected individuals was used in the experiments with minimal \textit{in vitro} passaging.\textsuperscript{15} Yet, synergy has been seen \textit{in vitro} between camostat mesylate and E-64D with SARS-CoV-2\textsuperscript{15}, suggesting that such synergy may also occur \textit{in vivo}.

We note that in addition to TMPRSS2 and Cathepsin B/L, with SARS-CoV-2, unlike SARS-CoV, the use of furin for S protein activation has been suggested.\textsuperscript{14,36,67,68} In a recent study, the role of furin in S protein activation of SARS-CoV-2 has been suggested to be similar to that in MERS-CoV, where furin cleaves S at the S1/S2 site, following which TMPRSS2 cleavage of S is required for entry through the fusion pathway.\textsuperscript{68} Thus, furin does not appear to replace TMPRSS2, but instead places an additional requirement, akin to ACE2 binding, for entry through the TMPRSS2 pathway. Further, eliminating the furin cleavage site did not affect entry through the Cathepsin B/L, or endocytosis, pathway. Our prediction of the synergy between TMPRSS2 and Cathepsin B/L inhibitors is thus independent of the role of furin.

In summary, our study identified unexpected synergy between TMPRSS2 and Cathepsin B/L inhibitors against SARS-CoV-2 infection, highlighted its existence in available \textit{in vitro} studies, and elucidated its novel origins at the single cell and cell population level using mathematical modeling. Our findings may have implications for the treatment of COVID-19 using agents blocking host proteases required for SARS-CoV-2 entry. Although we focussed on TMPRSS2 and Cathepsin B/L, our findings apply more broadly to combinations of agents that block the two independent pathways, fusion and endocytosis, of SARS-CoV-2 entry into target cells.
Methods

Model of SARS-CoV-2 entry via TMPRSS2 and Cathepsin B/L mediated pathways

We considered in vitro experiments where target cells expressing TMPRSS2 and Cathepsin B/L were exposed to virions or pseudo-typed viral particles bearing the spike protein, S. Experiments were performed in the absence or presence of inhibitors of TMPRSS2 and Cathepsin B/L and the fraction of infection events measured in 16 to 24 h post virus exposure. We developed the model below to describe the ensuing viral dynamics.

Viral dynamics. We recognized that the expression levels of the proteases would vary across cells and affect viral entry into the cells. Following previous approaches\textsuperscript{46,52}, we divided the cells into subpopulations based on the expression levels of the proteases. We defined $T_c$ as the subpopulation of target cells expressing TMPRSS2 in a narrow range $\Delta n_t$ around $n_t$ copies per cell and Cathepsin B/L in a narrow range $\Delta n_c$ around $n_c$ copies per cell. We described the relative susceptibility of these cells to virus entry through the TMPRSS2 pathway, $S_t$, and the Cathepsin B/L pathway, $S_c$, using Hill functions $S_t = \frac{(n_t)^{h_t}}{(n_t^{50})^{h_t} + (n_t)^{h_t}}$ and $S_c = \frac{(n_c)^{h_c}}{(n_c^{50})^{h_c} + (n_c)^{h_c}}$, where $h_t$ and $h_c$ were the Hill coefficients, and $n_t^{50}$ and $n_c^{50}$ were receptor expression levels at which $S_t = 0.5$ and $S_c = 0.5$, respectively. Such Hill functional forms have been used to describe the effects of other proteases.\textsuperscript{69} The overall susceptibility of the cells, due to entry through both the pathways, was $S_{\text{ec}} = S_t + S_c - S_t S_c$. In the presence of a TMPRSS2 inhibitor, a Cathepsin B/L inhibitor, or both, the susceptibilities were lowered to $S_{\text{ec}}(D_T)$, $S_{\text{ec}}(D_C)$, and $S_{\text{ec}}(D_T, D_C)$, respectively, where $D_T$ and $D_C$ were the concentrations of the TMPRSS2 and Cathepsin B/L inhibitors (see below). The following equations described the ensuing viral infection dynamics:
\[
\frac{dT_{tc}}{dt} = (\lambda - \mu)T_{tc} - kS_{tc} T_{tc} V
\]  
(1)

\[
\frac{dI_{tc}}{dt} = kS_{tc} T_{tc} V - \delta I_{tc}
\]  
(2)

\[
\frac{dV}{dt} = -cV
\]  
(3)

Here, target cells proliferate and die with rate constants \(\lambda\) and \(\mu\), respectively. \(k\) is the second order rate constant of the infection by free virions, \(V\), of target cells expressing excess TMPRSS2, Cathepsin B/L, or both, so that entry is not limited by the proteases, \(i.e.,\) for cells with the susceptibility \(S_{tc} = 1\). \(k\) is reduced for cells \(T_{tc}\) by the factor \(S_{tc}\), the relative susceptibility. Successful infection leads to the production of the corresponding infected cells \(I_{tc}\). We neglected the proliferation of infected cells following previous models\(^{52,70}\). Infected cells die with a rate constant \(\delta\). Free virions are cleared with the rate constant \(c\). We focussed on experiments that lasted \(\sim24\) h or less after exposure of the cells to virions, by which time it is unlikely that sizeable numbers of progeny virions are produced from infected cells. We therefore neglected progeny virion production. The model would apply naturally to experiments with pseudo-typed viruses, with \(I_{tc}\) representing cells into which successful virus entry has occurred.

**Distribution of protease expression levels across cells.** We assumed that at the start of infection, the fraction, 
\[
\phi_{tc} = \psi_{tc}(n_i, n_c) \Delta n_i \Delta n_c,
\]

of cells belonging to the subpopulation \(T_{tc}\) was determined by the joint distribution,
\[
\psi_{tc}(n_i, n_c) = \psi_i(n_i) \psi_c(n_c), \text{ where } \psi_i(n_i) = \frac{1}{n_i \sigma_i \sqrt{2\pi}} e^{-\frac{(\ln n_i - \bar{n}_i)^2}{2\sigma_i^2}}, \text{ and } \psi_c(n_c) = \frac{1}{n_c \sigma_c \sqrt{2\pi}} e^{-\frac{(\ln n_c - \bar{n}_c)^2}{2\sigma_c^2}} \text{ were the independent log-normal distributions of the expression levels of TMPRSS2 and Cathepsin B/L, respectively,}
\]

across cells with \(\bar{n}_i\) and \(\bar{n}_c\) the associated means, and \(\sigma_i\) and \(\sigma_c\) the standard deviations. We divided the target cells into \(M \times N\) subpopulations, so that \(i = 1, 2, \ldots, M\) and \(c = 1, 2, \ldots, N\).

**Effect of drugs.** We assumed that drugs bound their target proteases and prevented their functioning. The susceptibility of cells would thus be reduced and be determined by the protease molecules unbound to the
inhibitors. In the presence of a TMPRSS2 inhibitor, we let the abundance of free TMPRSS2, denoted \( n_t^f \), follow \( n_t^f = \frac{\gamma_T}{\gamma_T + D_T} n_t \). The susceptibility of cells \( T_{tc} \) to infection through the TMPRSS2 pathway reduced to

\[
S_t(D_T) = \frac{(n_t^f)^h}{(n_t^{50})^h + (n_t^f)^h}, \quad \text{and the overall susceptibility became } S_{tc}(D_T) = S_t(D_T) + S_c - S(D_T)S_c.
\]

Similarly, in the presence of a Cathepsin B/L inhibitor, we let the abundance of free Cathepsin B/L, \( n_c^f \), available for mediating virus entry, follow \( n_c^f = \frac{\gamma_C}{\gamma_C + D_C} n_c \). The susceptibilities accordingly reduced to

\[
S_c(D_C) = \frac{(n_c^f)^h}{(n_c^{50})^h + (n_c^f)^h}, \quad \text{and } S_{tc}(D_T, D_C) = S_t(D_T) + S_c(D_C) - S(D_T)S_c(D_C).
\]

With both the drugs used simultaneously, the susceptibilities were \( S_{tc}(D_T, D_C) = S_t(D_T) + S_c(D_C) - S(D_T)S_c(D_C) \). Here, \( D_T \) was the concentration of the TMPRSS2 inhibitor, \( D_C \) that of the Cathepsin B/L inhibitor, and \( \gamma_T \) and \( \gamma_C \) were the inhibition constants representing drug levels that reduced the respective free protease levels by half. These expressions for the free protease numbers followed dose-response relationships that could be derived mechanistically assuming reaction equilibrium of drug-protease binding and species balance constraints\(^{52}\).

**Synergy.** We solved Eqns. [1-3] to predict the number of infected cells at day 1 post-infection in each subpopulation in absence of drugs, denoted \( I_{tc} \), in the presence of a TMPRSS2 inhibitor, \( I_{tc}(D_T) \), in the presence of a Cathepsin B/L inhibitor, \( I_{tc}(D_C) \), and in the presence of both inhibitors, \( I_{tc}(D_T, D_C) \). From these populations, we estimated the fraction unaffected by the drugs in the respective subpopulations as the ratios,

\[
f_{tc}^u(D_T) = \frac{I_{tc}(D_T)}{I_{tc}}, \quad f_{tc}^u(D_C) = \frac{I_{tc}(D_C)}{I_{tc}} \quad \text{and } f_{tc}^u(D_T, D_C) = \frac{I_{tc}(D_T, D_C)}{I_{tc}}.
\]

From these fractions, we estimated the fraction that would be uninhibited assuming Bliss independence, \( f_{tc, Bliss}^u(D_T, D_C) = f_{tc}^u(D_T)f_{tc}^u(D_C) \), and the extent of Bliss synergy, \( \beta_{Bliss}^{tc} = f_{tc, Bliss}^u(D_T, D_C) - f_{tc}^u(D_T, D_C) \). This yielded the extent of synergy within the cell subpopulation and thus indicated synergy arising from the effects of the drugs at the single cell level. We
estimated the total single cell level synergy by averaging across all subpopulations: \( \beta_{\text{cell}}^{\text{Biss}} = \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} \beta_{tc}^{\text{Biss}} \). In other words,

\[
\beta_{\text{cell}}^{\text{Biss}} = \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} \left[ \frac{I_{tc}(D_T) \times I_{tc}(D_C) - I_{tc}(D_T, D_C)}{I_{tc}} \right]
\]

Identifying the synergy at the cell population level was more involved. It required deriving ways to decouple single cell level synergy from the cell population level synergy. We accomplished it by considering single round infection assays, which we describe below. Accordingly, the cell population synergy was then given by the following expression:

\[
\beta_{\text{popn}}^{\text{Biss}} = \frac{1}{\sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} (S_t - S_t (D_T))(1-S_c) \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} (S_c - S_c (D_C))(1-S_t)} \left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} (S_t - S_t (D_T))(1-S_c) \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_{tc} (S_c - S_c (D_C))(1-S_t) \right]
\]

Finally, we computed the overall Bliss synergy as follows. From the expressions above, the total fractions unaffected by the drugs individually and together were written as \( f_{I}(D_T) = \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_T)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} \), \( f_{II}(D_C) = \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_C)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} \), and \( f_{II}(D_T, D_C) = \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_T, D_C)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} \). The expected fraction unaffected assuming Bliss independence was thus \( f_{\text{Biss}}^{I}(D_T, D_C) = f_{I}(D_T)f_{I}(D_C) \), and the extent of Bliss synergy,

\[
\beta_{\text{Biss}} = f_{\text{Biss}}^{I}(D_T, D_C) - f_{II}(D_T, D_C) \). Thus,

\[
\beta_{\text{Biss}} = \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_T)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} \times \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_C)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} - \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_T, D_C)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}} \times \frac{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}(D_T, D_C)}{\sum_{c=1}^{N} \sum_{t=1}^{M} I_{tc}}
\]

Model of single round infection assays
We considered experiments where virions, such as those pseudo-typed with SARS-CoV-2 S protein, were capable of infecting cells but not producing progeny virions, were used, restricting infection to a single round. We derived expressions to estimate the synergy arising from the single cell level and the cell population level as well as the total synergy as follows.

**Synergy at the single cell level.** To describe the synergy arising at the single cell level, we considered the subpopulation $T_{ic}$. We employed the definitions of the susceptibilities $S_{ic}$ above. In a single round assay, the fractions of cells infected in the absence and presence of drugs, on average, would equal the susceptibility of the cells. Thus, we could write $I_{ic} = T_{ic} S_{ic} = T_{ic} (S_i + S_c - S_{ic})$, $I_{ic}(D_T) = T_{ic} S_{ic}(D_T)$, $I_{ic}(D_c) = T_{ic} S_{ic}(D_c)$, and $I_{ic}(D_T, D_c) = T_{ic} S_{ic}(D_T, D_c)$. Employing these expressions in the definitions of the fractions unaffected by the drugs, $f_{ic}^u(D_T) = \frac{I_{ic}(D_T)}{I_{ic}}$, $f_{ic}^u(D_c) = \frac{I_{ic}(D_c)}{I_{ic}}$ and $f_{ic}^u(D_T, D_c) = \frac{I_{ic}(D_T, D_c)}{I_{ic}}$, and simplifying, we estimated the Bliss synergy, $\beta_{Bliss}^ic = f_{ic}^u(D_T) f_{ic}^u(D_c) - f_{ic}^u(D_T, D_c)$, which upon simplifying yielded

$$\beta_{Bliss}^ic = \frac{(S_i - S_{ic}(D_T))(S_i - S_{ic}(D_c))}{(S_i + S_c - S_{ic})^2}.$$ 

The total synergy from the single cell level followed from averaging the above expression across all the cell subpopulations:

$$\beta_{Bliss}^{cell} = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} \beta_{Bliss}^ic = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} \frac{(S_i - S_{ic}(D_T))(S_i - S_{ic}(D_c))}{(S_i + S_c - S_{ic})^2}. \quad (7)$$

**Overall synergy.** We next considered the overall synergy, arising from both the single cell and the cell population level. For this, we estimated the total fractions of cells infected in the absence and presence of drugs, recognizing that in each subpopulation, the fraction would equal the corresponding susceptibility. We thus wrote:

$$f_{\text{inf}} = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} f_{ic}^\text{inf}, \quad f_{\text{inf}}(D_T) = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} f_{ic}^\text{inf}(D_T), \quad f_{\text{inf}}(D_c) = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} f_{ic}^\text{inf}(D_c), \quad \text{and}$$

$$f_{\text{inf}}(D_T, D_c) = \sum_{c=1}^{N} \sum_{\tau=1}^{M} \phi_{ic} f_{ic}^\text{inf}(D_T, D_c),$$

where the fractions infected within the subpopulations were $f_{ic}^\text{inf} = S_{ic}$, $f_{ic}^\text{inf}(D_T) = S_{ic}(D_T)$, $f_{ic}^\text{inf}(D_c) = S_{ic}(D_c)$, and $f_{ic}^\text{inf}(D_T, D_c) = S_{ic}(D_T, D_c)$. The total fractions unaffected by the
drugs were thus \( f^u(D_T) = \frac{f^{\text{inf}}(D_T)}{f^{\text{inf}}} \), \( f^u(D_C) = \frac{f^{\text{inf}}(D_C)}{f^{\text{inf}}} \), and \( f^u(D_T, D_C) = \frac{f^{\text{inf}}(D_T, D_C)}{f^{\text{inf}}} \). The extent of Bliss synergy was then \( \beta_{\text{Bliss}} = f^u(D_T)f^u(D_C) - f^u(D_T, D_C) \). Substituting the expressions derived above for the fractions infected in terms of the susceptibilities, performing algebraic manipulations, and simplifying yielded

\[
\beta_{\text{Bliss}} = \frac{1}{\left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c + S_c - S_t, S_t) \right]^2} \left[ \left( \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c - S_t(D_T))(1-S_t) \right) \left( \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c - S_t(D_C))(1-S_t) \right) \right] - \left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c^2(S_c - S_t(D_T))(S_c - S_t(D_C)) \right] \left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c + S_c - S_t, S_t) \right].
\]

\( \text{(8)} \)

**Synergy at the cell population level.** Deriving expressions for the synergy at the cell population from first principles was not straightforward. We therefore employed the following route. We recognized that the total synergy was a convolution of the synergy at the single cell and the cell population levels. We thus reasoned that the expression for the total synergy above would reduce to the synergy at the cell population level in the scenario where no single cell level synergy existed. The lack of synergy at the single cell level implied

\[
\beta_{\text{Bliss}}^c = (S_c - S_t(D_T))(S_c - S_t(D_C)) / (S_c + S_c - S_t, S_t)^2 = 0.
\]

Accordingly, the second term in the numerator of the expression for the overall synergy, \( \beta_{\text{Bliss}} \), above dropped. Further, in the first term, the elements corresponding to the same values of \( t \) and \( c \) in the two summations also dropped. The resulting expression could thus be written as,

\[
\beta_{\text{Bliss}}^{\text{popn}} = \frac{1}{\left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c + S_c - S_t, S_t) \right]^2} \left[ \left( \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c - S_t(D_T))(1-S_t) \right) \left( \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c(S_c - S_t(D_C))(1-S_t) \right) \right] - \left[ \sum_{c=1}^{N} \sum_{t=1}^{M} \phi_c^2(S_c - S_t(D_T))(1-S_t)(S_c - S_t(D_C))(1-S_c) \right],
\]

yielding the synergy at the cell population level from single round assays. The expression is listed above as Eq. (5). (Note that the second term in the numerator here is to eliminate terms from the first term that have the same values of \( t \) and \( c \) in the two summations.)

We verified that the latter expression reduced to the cell population level synergy that we derived from first principles in the limiting scenario where only two subpopulations existed, one expressing TMPRSS2 and the other Cathepsin B/L. For this, we let the first subpopulation express TMPRSS2 and no Cathepsin B/L and
the second vice versa. We thus let \( t \in \{1, 2\} \) and \( c \in \{1, 2\} \) and set \( \phi_{11} = \phi_t, \ \phi_{22} = \phi_c = 1 - \phi_t, \) and \( \phi_{12} = \phi_{21} = 0. \) Thus, \( T_{11} \) and \( T_{22} \) were the subpopulations expressing TMPRSS2 alone and Cathepsin B/L alone, respectively.

We let their susceptibilities in the absence of drugs be \( S_{11} = S_t \) and \( S_{22} = S_c \). In the presence of a TMPRSS2 inhibitor, the susceptibility \( S_{11}(D_T) = S_t(D_T) \), whereas \( S_{22} \) would remain unchanged. The opposite happened with a Cathepsin B/L inhibitor. With this description, the expression above for \( \beta_{\text{Bliss}}^{\text{pops}} \) reduced to

\[
\beta_{\text{Bliss}}^{\text{pops}} = \frac{\phi_t (S_t - S_t(D_T))(S_c - S_c(D_c))}{\left[ \phi_t S_t + \phi_c S_c \right]^2},
\]

identical to the expression we derived from first principles (see Text).

We therefore employed the above expression for \( \beta_{\text{Bliss}}^{\text{pops}} \) (Eq. (5)) to estimate the cell population level synergy in our heterogeneous cell population. The expression, however, was restricted to single round assays. Decoupling the two synergies in multiple round assays proved difficult.

Data

We considered experiments were different cell lines are exposed to SAR-CoV or SARS-CoV2 pseudotyped virions in the presence or absence of TMPRSS2 and Cathepsin B/L inhibitors, and the fraction of infection events unaffected by the drugs was estimated.\(^{15,19}\) We extracted the data reported using Engauge Digitizer 12.1 and estimated the extent of synergy using the Bliss index. The cell lines and drugs used are mentioned in the Results section.

Model calculations and parameters.

The model equations were solved using programs written in MATLAB (MathWorks, Natick, MA) and Mathematica. The model parameters used have been chosen, where possible, to apply to SARS-CoV-2 infections in vitro. Others are drawn from studies on other viral infection kinetics in vitro. Some unknown parameters had to be assumed but their values did not affect our findings. Briefly, we let \( \lambda = 0.77 \) per day\(^{71}; \\mu = 0.22 \) per day\(^{72}; \ \delta = 0.53 \) per day\(^{49}; c = 10 \) per day\(^{49}; k = 1 \times 10^{-4} \) ml per ffu per day\(^{46}; h_t = h_c = 4^{46}; \ \sigma_t = \sigma_c.\)
and the endpoint of the experiment, $t_d = 24$ hours\(^\text{15}\). We assumed the following parameter values: $n_i = n_c = 11.5$; $n_i^{50} = 1.2 \times 10^5$ copies/cell; and $n_c^{50} = 2.4 \times 10^5$ copies/cell. We let distribution of the protease expression levels be discretised with $\Delta n_i = \Delta n_c = 0.2$. Finally, we chose the following initial conditions: $T(0) = 1 \times 10^5$ cells per ml\(^46\) and $V(0) = 1 \times 10^4$ ffu per ml\(^46\). Any deviations from these values are mentioned in the figure captions.

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**Conflicting interests**

The authors declare that no conflicts of interests exist.

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