Modeling Suggests a Mechanism of Synergy Between Hepatitis C Virus Entry Inhibitors and Drugs of Other Classes

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Hepatitis C virus (HCV) entry inhibitors (EIs) act synergistically with drugs targeting other stages of the HCV lifecycle. The origin of this synergy remains unknown. Here, we argue that the synergy may arise from the complementary activities of the drugs across cell subpopulations expressing different levels of HCV entry receptors. We employ mathematical modeling of viral kinetics in vitro, where cells with a distribution of entry receptor expression levels are exposed to HCV with or without drugs. The drugs act independently in each cell, as expected in the absence of underlying interactions. Yet, at the cell population level our model predicts that the drugs exhibit synergy. EIs effectively block infection of cells with low receptor levels. With high receptor levels, where EIs are compromised, other drugs are potent. This novel mechanism of synergy, arising at the cell population level may facilitate interpretation of drug activity and treatment optimization.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? Strong synergy has been observed between the numerous inhibitors targeting hepatitis C virus (HCV) entry and antiviral agents targeting other stages of the HCV lifecycle. The origin of the synergy remains poorly understood.

WHAT QUESTION DID THIS STUDY ADDRESS? We constructed a mathematical model of HCV viral kinetics in vitro to unravel the mechanism of synergy between the drugs.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE Our model demonstrates that heterogeneity of entry receptor expression levels across cells, leading to complementary activity of drugs across different cell subpopulations, can give rise to the observed synergy between the HCV entry inhibitors and drugs of other classes. Synergy may thus arise not only from molecular/intracellular level interactions between drugs but also from heterogeneities at the cell population level.

 HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS The mechanism of synergy that we have identified may enable more accurate interpretation of drug efficacies in combination and facilitate rational optimization of treatment with HCV entry inhibitors.

Hepatitis C virus (HCV) entry into target cells involves the viral envelope proteins E1 and E2 and several cell surface receptors including scavenger receptor class B type 1 (SR-B1), CD81, tight-junction proteins claudin-1 (CLDN1) and occludin (OCLN), Niemann-Pick C1-Like 1 (NPC1L1), and transferrin receptor 1 (Tfr1). Additionally, host factors such as epidermal growth factor receptor (EGFR), ephrin receptor A2 (EphA2), and HRas have been shown to modulate HCV entry. Entry inhibitors (EIs) targeting viral envelop proteins or host receptors have been able to prevent infection in vitro and in a mouse model and present a promising new class of anti-HCV drugs. Indeed, two EIs, ITX 5061, which targets SR-B1, and erlotinib, which targets EGFR, are in clinical trials and several compounds targeting other aspects of the HCV entry process are in development.

Recent studies have observed strong synergy between EIs and other classes of direct-acting antiviral agents (DAAs) or host-targeting agents (HTAs) that are in use or in clinical development. The EIs investigated in these studies are monoclonal antibodies targeting CD81, CLDN1, or SR-B1, and small molecule inhibitors targeting SR-B1 (ITX 5061), EGFR (erlotinib), or EphA2 (dasatinib). The DAAs investigated are protease inhibitors (telaprevir, boceprevir, simeprevir, and danoprevir), an NS5A inhibitor (daclatasvir), and polymerase inhibitors (sofosbuvir and mericitabine). The HTAs investigated are a cyclophilin inhibitor (alisporivid) and pegylated interferon α.

Synergy implies that in combination lower drug dosages can yield the desired efficacy. Consequently, unraveling the mechanism(s) underlying this observed synergy assumes importance: it would facilitate the rational identification of optimal drug dosages that would maximize treatment response, yielding guidelines for the use of EIs in combination with other drugs. One possibility that may give rise to synergy between an EI and another drug is the existence of an interaction, as yet unknown, between the HCV entry process and the step of the HCV lifecycle targeted by the other drug. For instance, if blocking one of the entry receptors were also to affect viral replication via signaling downstream of the entry receptor, then synergy between EIs and viral polymerase inhibitors may arise. Such a possibility, however, appears unlikely because EIs display synergy with drugs from several classes and interactions between each
Figure 1 Schematic of the hypothesis. The expression level of an entry receptor varies across cells (left). The entry inhibitor blocks the infection of cells with low receptor expression, whereas a DAA or an HTA of another class is more effective in blocking the infection of cells with high receptor expression (right). This complementary activity of the drugs across subpopulations of cells may give rise to synergy.

of the steps of the HCV lifecycle targeted by the latter drugs and the viral entry process are not foreseen. Another possibility is that EIs act on strains that carry resistance mutations to the other drug, thus increasing the overall genetic barrier of the combination. EIs, however, also exhibit synergy with interferon, which acts by stimulating the host-immune response, and against which resistant strains are not expected to arise. Thus, while the above mechanisms might contribute to synergy in specific cases, a more general mechanism appears to underlie the broadly observed synergy between EIs and other drugs.

Here we explore an alternative hypothesis that could explain the broad synergy between EIs and other drugs. We argue that synergy between an EI and another drug could arise from the complementary activities of the two drugs across cell subpopulations expressing different levels of entry receptors (Figure 1). By entry receptors we mean the cell or viral surface proteins or other host factors listed above that affect HCV entry. A distribution of the expression level of entry receptors is typically observed across cells in culture.19 Entry efficiency increases with receptor expression.19,20 Blocking virus entry into cells with higher receptor expression levels therefore requires larger dosages of the EI. Infection of such cells, however, can be blocked more readily by the other drug, which is unaffected by the efficiency of the entry process. Thus, when used in combination, the EI need only target cells with low entry receptor expression levels, which is accomplished at lower dosages, leading to the observed synergy.

To test this hypothesis, we constructed two mathematical models: a conceptual model that serves to elucidate the key underpinnings of the hypothesis, and a more comprehensive model that mimics experiments. We found, interestingly, that although, as expected, the drugs acted independently at the single-cell level, they displayed synergy at the cell-population level and that the extent of synergy increased with increase in the heterogeneity of entry receptor expression levels across cells.

METHODS

Conceptual model

Framework. We first constructed a simple, conceptual model where target cells, T, consisted of only two subpopulations of cells expressing low and high levels of entry receptors. The expression level of entry receptors is typically observed across cells in culture.19 Entry efficiency increases with receptor expression.19,20 Blocking virus entry into cells with higher receptor expression levels therefore requires larger dosages of the EI. Infection of such cells, however, can be blocked more readily by the other drug, which is unaffected by the efficiency of the entry process. Thus, when used in combination, the EI need only target cells with low entry receptor expression levels, which is accomplished at lower dosages, leading to the observed synergy.

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Mathematical model

Figure 2a Schematic of the hypothesis. The level of entry receptors is typically observed across cells in culture.19 Entry efficiency increases with receptor expression.19,20 Blocking virus entry into cells with higher receptor expression levels therefore requires larger dosages of the EI. Infection of such cells, however, can be blocked more readily by the other drug, which is unaffected by the efficiency of the entry process. Thus, when used in combination, the EI need only target cells with low entry receptor expression levels, which is accomplished at lower dosages, leading to the observed synergy.
The dependence of susceptibility to infection on entry receptor expression, $S_i$, increases and the efficacy of entry inhibitors, $e_i$, decreases with increase in the receptor expression level. The other symbols employed are defined in the Methods.

Using $e_1$ and $e_2$ thus obtained, Eqs. 1–3 can be solved to predict viral kinetics under exposure to any drug levels $C_E$ and $C_A$ given the initial distribution of the cell population into the two subpopulations. We assumed that at the start of the infection a fraction $\phi$ of the target cells is of type $T_2$. We varied $\phi$ between 0 and 1. Further, we assumed that infection was seeded by free virions and no infected cells existed at the start.

Synergy. The predictions of viral kinetics were employed to assess the synergy between the drugs. This involved solving the inverse problem of identifying drug concentrations for a desired level, $x$, of inhibition of infection at a fixed timepoint during the course of infection, with the inhibition measured in terms of the fraction of cells infected. We first fixed $x$ to, say, 50%. We solved the model equations above first without drugs and predicted the number of infected cells at the desired timepoint, say, 3 days postinfection. We next solved the equations in the presence of the EI alone and identified that concentration $C_{E,x}$ at which the number of infected cells was lowered by the factor $x$ at the desired timepoint. We repeated the procedure with the other drug alone and identified the corresponding concentration $C_{A,x}$. We finally solved the equations with the drugs used simultaneously and identified the concentrations $C_E$ and $C_A$ at
which the same level of inhibition was achieved. To minimize our search space, following experiments,9,18 we let the ratio of $C_E$ and $C_A$ be equal to the ratio of $(C_E)_x$ and $(C_A)_x$, i.e., $C_E/C_A = (C_E)_x/(C_A)_x$. The combination index,

$$CI_x = \frac{C_E}{(C_E)_x} + \frac{C_A}{(C_A)_x}, \quad (7)$$

then yielded the extent of synergy (or antagonism) between the drugs at the level of inhibition considered. (Use of the alternative expression, $CI_x = \frac{C_E}{(C_E)_x} + \frac{C_A}{(C_A)_x} + \frac{C_E + C_A}{(C_E)_x + (C_A)_x}$, did not alter our findings; see Supplementary Figure 1.) We repeated the procedure for different values of $x$ (=50%, 75% and 90%) as in the experiments.9,18

**Model of HCV viral kinetics in vitro Framework.** We next considered in vitro experiments where a population of target cells is exposed to a population of HCV virions with or without drugs (Figure 2b). The cell population was assumed to exhibit a distribution of the expression level of the entry receptor above. We therefore divided the target cells into $K$ subpopulations, denoted by $T_i$, where $i = 1, 2, \ldots, K$, with cells $T_i$ expressing the entry receptor in a narrow range $\Delta n_i$ around $n_i$ molecules per unit area. The following equations describe the ensuing viral kinetics:

$$dT_i/dt = \lambda \left(1 - \frac{\sum_{j=1}^{K} T_j}{T_{\text{max}}}\right) T_i - \mu T_i - \beta (1 - \xi_i) S_i T_i V, \quad i = 1, 2, \ldots, K \quad (8)$$

$$dV_i/dt = \beta (1 - \xi_i) S_i T_i V - \delta_i V_i, \quad i = 1, 2, \ldots, K \quad (9)$$

$$dV_i/dt = p \sum_{j=1}^{K} I_j - cV \quad (10)$$

Here, $\lambda$, $\mu$, $\delta$, $p$, and $T_{\text{max}}$ are the same as in Eqs. 1–3. We additionally recognized that the efficiency of entry increases with the expression level of the entry receptor, $n_i$. We modeled this efficiency using the relative susceptibility $S_i$ of cells $T_i$ to virus entry. Based on previous studies,20,26 we let $S_i$ depend on entry receptor following a Hill function, $S_i = \frac{(n_i)^h}{(n_i)^h + (n_i)^0}$, where $h$ is the Hill coefficient and $n_i^0$ is the entry receptor expression level at which $S_i = 0.5$. Note that when $n_i \approx 0$, $S_i \approx 0$ and when $n_i \gg n_i^0$, $S_i \approx 1$. $\beta$ is thus the infection rate of cells expressing the entry receptor in excess.

**Drug efficacies.** Here we adopted a more mechanistic approach to evaluate the efficacy of the EI, based on the assumption that it targeted the entry receptor that distinguished the different cell subpopulations. (Note that the empirical approach based on the Hill function employed in the conceptual model above can also be used here.) In the presence of the inhibitor, thus, the number of free receptors, $n_i^f$, available for HCV entry decreased due to the formation of the receptor–inhibitor complexes, $n_i^c$. The susceptibility of cells $T_i$ to infection in the presence of the EI consequently reduced to $S_i^d = \frac{(n_i/n_i^c)^h}{(n_i/n_i^c)^h + (n_i/n_i^0)^h}$. Assuming complex formation to be rapid, we estimated the number of complexes formed using reaction equilibrium to be $n_i^c = \frac{K_E}{K_E + K_D}$, where $K_D$ is the equilibrium dissociation constant of the complexes. Mass balance on the receptor expression level, $n_i = n_i^f + n_i^c$, then yielded $n_i^f = \frac{C_E}{(C_E)_x + C_A} n_i$ and $n_i^c = \frac{C_A}{(C_A)_x}$. Substituting the latter expression for $n_i^c$ in the expression above yielded $S_i^d$. We recognized next that $S_i^d = S_i (1 - \xi_i)$, where $\xi_i$ is the efficacy of the EI in cells $T_i$ when used alone. Using the expression for $S_i^d$, combining it with the expression for $S_i$ and rearranging terms yielded $\xi_i$ as a function of the receptor expression level, $n_i$:

$$\xi_i = \left(1 + \frac{C_E}{(C_E)_x}\right)^{-\frac{1}{h}} - 1$$

By letting $\xi_i = 0.5$, we obtained from the above expression the concentration of the EI required to block the infection of $T_i$ by 50%: $IC^E_{50} = K_E^E ((2 \pm (\frac{1}{h})^h - 1)$. Like in the conceptual model, the other drug was assumed to block the infection of cells in each subpopulation with efficacy $\xi_{\text{CA}} = \frac{C_A}{(C_A)_x}$, independently of the expression level of the “entry” receptor. (Relaxing this assumption by allowing the latter efficacy also to exhibit an independent distribution across cells led to greater synergy; see Supplementary Text, Supplementary Figure 2.) In the presence of the EI alone, $\xi_i = \xi_i^E$. In the presence of the other drug alone, $\xi_i = \xi_i^A$. In the presence of both the drugs at concentrations $C_E$ and $C_A$, respectively, the combined efficacy, $\xi_{\text{CA}}$, can be obtained by solving the equation below, derived following the same procedure employed for Eq. 5, i.e., by enforcing $CI = 1$:

$$\frac{C_E}{\left(\frac{1}{n_i^0} + \frac{n_i^0}{1-\xi_i}\right)^{1/h}} + \frac{C_A}{IC^A_{50} \left(\frac{1}{n_i^0} - 1\right)^{1/h/m_A}} = 1$$

Knowledge of the efficacies, $\xi_i$, allowed us to solve Eqs. 8–10 and predict the resulting viral kinetics for any $C_E$ and $C_A$ given an initial distribution of cells in the various subpopulations.

**Synergy.** To estimate synergy, we followed the same procedure employed in the conceptual model above, where we first identified the concentrations $(C_E)_x$ and $(C_A)_x$ of the EI and the other drug required for achieving a desired level of inhibition, $x$, when used alone. We then determined the concentrations $C_E$ and $C_A$ at which the same level of inhibition was achieved when the drugs were used together. $CI$, evaluated using Eq. 7, then provided a measure of synergy.

**Data.** We considered data from previous cell culture studies where human hepatoma-derived cells were exposed to cell culture-derived HCVcc virions in the presence of EIs and/or
Drug concentrations are normalized by the respective in vitro fraction of infection events prevented by the drug. The corresponding median effect equation, 

\[ T(\text{d}) = \frac{IC_{50}}{C_1} \]

and infected cells, blocking the infection of cells the ratio of the number of infection events inhibited by the drug to the number uninhibited. (c) The efficacy of a DAA or an HTA in blocking the infection of cells \( T_1 \) and \( T_2 \), respectively, for different drug concentrations, \( C_A \), and the corresponding median-effect plot. The efficacy of the combination in blocking the infections of \( T_1 \) and \( T_2 \) and infected cells, the fraction of cells expressing high receptor expression levels, \( T_2 \), and the viral titer, \( V \), the ratio of \( IC_{50} \) and \( IC_{90} \). Drug concentrations are normalized by the respective \( IC_{50} \) values. Parameter values: \( \lambda = 0.44 \text{ d}^{-1} \), \( \mu = 1.7 \times 10^{-5} \text{ d}^{-1} \), \( \delta = 1.1 \times 10^{-2} \text{ d}^{-1} \), \( \beta = 1.2 \times 10^{-4} \text{ ml (ffu-d)}^{-1} \), \( \rho = 2.78 \text{ ffu (ml-d)}^{-1} \), and \( c = 23.2 \text{ d}^{-1} \). We assumed \( T_{\text{max}} = 7 \times 10^6 \text{ cells ml}^{-1} \), representative of in vitro cultures. \(^{16} \) The Hill coefficients, \( m_a = 1 \), \( m_{E_1} = 1 \) and \( m_{E_2} = 1 \). Initial conditions: target cells, \( T(0) = 10^6 \text{ cells ml}^{-1} \), with \( T_1(0) = (1 - \phi) T(0) \) and \( T_2(0) = \phi T(0) \); virions, \( V(0) = 10^5 \text{ ffu ml}^{-1} \) (ffu stands for focus-forming units); and infected cells, \( I_i(0) = 0 \).

### Results

**DAAs and HTAs of other classes.** In these experiments, target cells proliferate, die, and get infected by HCVcc virions. Free virions are in turn produced by infected cells and are lost due to natural degradation. Viral infection was assessed using luciferase activity and CI was calculated at 50, 75, and 90% inhibition to assess synergy. \(^{9,18} \)

**Calculations and model parameters**

We solved the model equations using computer programs written in MatLab (MathWorks, Natick, MA) and/or Berkeley Madonna (www.berkeleymadonna.com) and computed the time-evolution of all of the subpopulations of uninfected cells, \( T_i \), infected cells, \( I_i \), and the viral titer, \( V \). The extent of inhibition was obtained as the ratio of the total population of infected cells, \( I = \sum_{i=1}^{K} I_i \), in the presence of drug(s) to that in the absence of drugs at day 3 postinfection. Using viral load instead of infected cells to quantify the extent of inhibition did not alter our findings (Supplementary Figure 3). We employed model parameters that described the kinetics of infection of Huh-7.5 cells with JFH1 virus\(^{20} \) (Figure 3). We also examined the sensitivity of our model predictions to variations in these parameter values (Supplementary Figure 4).

**RESULTS**

**Conceptual model**

To elucidate the role of heterogeneity in receptor expression in the observed synergy between an EI and a drug of another class, we constructed a conceptual model where target cells in culture expressed one of two discrete levels (high or low) of one entry receptor and were identical otherwise (Figure 2a: Methods). Within each cell, and hence subpopulation, we assumed that the drugs acted independently and examined whether they exhibited synergy at the cell population level by computing the combination
index ($CI$). $CI < 1$, $CI = 1$ and $CI > 1$ indicate synergy, additivity, and antagonism, respectively.

**Efficacy of individual drugs.** In the two cell subpopulations, denoted $T_1$ and $T_2$, the EI acted with different efficacies. The difference in the receptor expression level between the two subpopulations was assumed to affect the $IC_{50}$: specifically, $IC_{E1}^{50} < IC_{E2}^{50}$, indicating greater efficacy of the inhibitor in the subpopulation $T_1$ (Figure 2a). The efficacies in the two subpopulations, denoted $e_{E1}$ and $e_{E2}$, respectively, increased with drug concentration in a sigmoidal manner (Figure 3a) (yielding a linear median-effect curve (Figure 3b). Further, as $IC_{E2}^{50}$ increased relative to $IC_{E1}^{50}$, higher drug levels were required to achieve the desired $e_{E2}$.

The dose–response curve of the other drug (a DAA or HTA of another class) also followed a sigmoidal pattern, but was the same for the two cell subpopulations (Figures 2a, 3c).

**Efficacy of drugs in combination.** We examined next the combined effect of the two drugs in each cell subpopulation. The independent activities of the drugs implied that in each subpopulation $CI = 1$. Thus, for a range of concentrations of the EI, $C_E$, and a fixed concentration of the other drug, $C_A$, we computed the net efficacy $e_1$ of the combination in the

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**Figure 4** Predictions of the model of HCV kinetics in vitro. (a) Dependence of the susceptibility of a cell to infection, $S_i$, on its receptor expression level, $n_i$. (b) Efficacy of the entry inhibitor, $e_{E1}$, and the other drug, $e_A$, and the combined efficacy of the two drugs, $e_1$, as functions of $n_i$. (c) The log-normal distribution, $f(n_i) = \frac{1}{\sqrt{2\pi}n_i\sigma_n} e^{-\frac{(\ln n_i - \mu)^2}{2\sigma_n^2}}$, of the receptor expression level across cells, where $\mu$ is the mean and $\sigma_n$ is the standard deviation of $\ln n_i$. (d) Combination index, $CI$, determined at 90% inhibition of the cumulative level of infection at day 3 postinfection for different values of $\sigma_n$. The concentrations of the entry inhibitor and the other drug are normalized by $K_{ED}^{E}$ and $IC_{A}^{50}$, respectively. (Note that $IC_{E}^{50}$ is directly proportional to $K_{ED}^{E}$; see Methods.) Parameters: $m_A = 1$, $h = 4$, $\eta = 2.9$ and $n_i^{10} = 1.65 \mu m^{-2}$. In (b) $CE = 54.77K_{ED}^{E}$ and $CA = 7.47IC_{A}^{50}$. Initial conditions: The fraction, $f(n_i)\Delta n_i$, of target cells belonging to the subpopulation $T_i$ follow the log-normal distribution above. The other parameters and initial conditions are the same as those listed in Figure 3.
subpopulation $T_1$, using Eq. 5 above. When $C_E$ was small, $e_1 \approx e_A$, the efficacy corresponding to $C_A$ alone (Figure 3d). As $C_E$ increased, $e_1$ rose in a sigmoidal manner and reached 1. For the subpopulation $T_2$, we found that much higher values of $C_E$ were required to achieve the same efficacies as in $T_1$ (Figure 3d). The activity of the EI was thus more prominent in the subpopulation $T_1$. We repeated the calculations for fixed $C_E$ and a range of values of $C_A$ (Figure 3e). In the subpopulation $T_1$, $e_1 \approx e_E$, the efficacy corresponding to $C_E$ alone, when $C_A$ was small and increased to 1 for large values of $C_A$. The trend was similar in the subpopulation $T_2$. However, the influence of the other drug was evident at much lower values of $C_A$ in $T_2$ than in $T_1$; $e_2$ began to rise above $e_{E1}$ at nearly 10-fold lower $C_A$ than when $e_1$ began to rise above $e_{E1}$. The influence of the other drug was thus more prominent in the subpopulation $T_2$.

Synergy. To describe the scenario when both types of cells, $T_1$ and $T_2$, were present in culture, we solved the viral kinetics equations (Eqs. 1–3) using the efficacies illustrated above, and estimated the drug levels required to achieve 90% inhibition of infection at day 3 postinfection. The drug levels then yielded $CI$ (via Eq. 7). We performed calculations over a range of values of $\phi$ ($0 \leq \phi \leq 1$), the fraction of cells of type $T_2$ at the start of infection, and different ratios of $IC_{50}^E$ and $IC_{50}^A$, denoted by $\alpha$ (Figure 3f and Supplementary Figure 5). When $\phi$ was either 0 or 1, a single cell type existed in the population and $CI$ equaled unity, indicating independence between the drugs. For intermediate values of $\phi$, where the cell population was heterogeneous, $CI$ was smaller than 1, indicating synergy. Similarly, when $\alpha$ was 1, indicating that the EI worked identically in the two subpopulations, heterogeneity was again lost and $CI = 1$ for all values $\phi$. As $\alpha$ decreased, indicating greater distinction between the two subpopulations, $CI$ decreased, indicating greater synergy between the drugs. Our model thus predicted synergy between the drugs ($CI < 1$) as arising from the heterogeneity of the underlying cell population and the resulting complementary activity of the drugs across cells.

With this conceptual understanding, we examined next whether a model that mimicked in vitro studies with cells exhibiting a continuous distribution of receptor expression levels also displayed synergy due to the same underlying principle.

Model of HCV viral kinetics in vitro

We divided target cells into several subpopulations with distinct receptor expression levels (Figure 2b) to mimic the distribution of receptor expression levels observed in cell culture studies. Further, we allowed entry efficiency to depend on receptor expression and estimated EI efficacy by quantifying its ability to block the entry receptor in question. The efficacy of the other drug remained independent of receptor expression. In each cell, the action of the two drugs was again assumed to be independent of each other. We estimated $CI$ based on a desired level of inhibition at different times postinfection to assess synergy at the cell population level (Methods).

Drug efficacies. The susceptibility of a cell to infection increased with receptor expression in a sigmoidal manner (Figure 4a). The efficacy of the EI correspondingly decreased with increase in receptor expression (Figure 4b). The efficacy of the other drug remained constant across subpopulations. We defined the combined efficacy of the drugs within each subpopulation, $e_i$, to be independent (by letting $CI = 1$). $e_i$ showed a dependence on receptor expression, $n_i$, as follows (Figure 4b). When $n_i$ was small, the EI was highly efficacious and more potent than the other drug. $e_i$ was then close to the efficacy of the EI. When $n_i$ was large, the EI was compromised, whereas the other drug continued to exert its antiviral activity. $e_i$ was then well approximated by the efficacy of the latter drug. At intermediate $n_i$, $e_i$ gradually switched between the two extremes (Figure 4b).

Heterogeneity in receptor expression and synergy. With the above estimations of $e_i$, we solved model equations (Eqs. 8–10) to predict the effect of drugs on HCV viral kinetics in vitro (Supplementary Figure 6) and estimated $CI$. (Note that the model equations are consistent with in vitro data of viral kinetics.20,26) We considered different levels of heterogeneity in the receptor expression level: as $\sigma_n$, the standard deviation of the log-normal distribution of receptor expression levels, increased, heterogeneity in receptor expression across cells increased (Figure 4c).

We found that $CI$ remained equal to 1 when the receptor expression across cells was homogeneous ($\sigma_n = 0$) (Figure 4d). This followed from the independence of the activities of the drugs in individual cells. We found, interestingly, that when the receptor expression across cells became heterogeneous ($\sigma_n > 0$), $CI$ became less than 1, suggesting synergy. $CI$ decreased as the heterogeneity in receptor expression ($\sigma_n$) increased (Figure 4d) as the complementary activity of the drugs at the population level became more prominent, consistent with our conceptual model (Figure 3f). $CI$ was largely insensitive to model parameters (Supplementary Figure 4) or the time postinfection when the assessment of synergy is made (Supplementary Figure 7). Our prediction of synergy due to heterogeneity in receptor expression is thus robust to changes in model parameter values.

DISCUSSION

Recent studies have shown strong synergy between ELs and DAAs or HTAs of other classes.9,18 Unraveling the mechanism of this observed synergy may facilitate optimization of combination treatments involving ELs. Mathematical models of HCV viral kinetics have been employed successfully to determine the effectiveness of treatment,24,27–29 identify mechanisms of the action of drugs,30,31 and to analyze patient data24,27 and cell culture experiments.20,26,32,33 In this study, advancing a previously developed mathematical model of HCV viral kinetics in vitro20,26 to account explicitly for drug action, we suggest that heterogeneity of receptor expression across cells and the resulting complementary action of drugs across distinct...
cell subpopulations may underlie the synergy between EIs and other drugs.

Synergy between drugs is thought to arise from an underlying interaction between the drugs or between the processes, pathways, and/or molecules they target. For instance, we demonstrated recently that DAAs targeting viral replication can alter the systems-level properties of the interferon signaling network and improve responsiveness of cells to type I interferon. Our current study presents an interferon signaling network and improve responsiveness of viral replication can alter the systems-level properties of the instance, we demonstrated recently that DAAs targeting targets in recent synergy between small molecule inhibitors of different HCV genotypes could thus explain at least in part the observed variations in the expression of entry receptor expression levels would provide tests of our proposed explanation.

HCV entry into target cells can occur by cell-free virions or cell-to-cell transmission. Although in our model we explicitly considered entry by cell-free virions, the model is applicable to entry via both modes. The viral titer is typically proportional to the population of infected cells, so that the infection rate constant in our model can be thought of as an effective rate constant for infection by both modes. We recognize, however, that if the EI considered blocks entry by one mode alone, then entry by the other mode can predominate, compromising drug efficacy and the resulting synergy. The mode of synergy elucidated by our study may have broader applicability. We anticipate this mode of synergy to arise whenever two drugs target different molecules that can predominate, compromising drug efficacy and the resulting synergy.

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