INTRODUCTION

The treatment of chronic hepatitis C virus (HCV) infection is undergoing a paradigm shift with the long-standing combination of pegylated interferon and ribavirin (PR) being replaced rapidly by combinations of new direct-acting antiviral agents (DAAs). The best DAA combinations today cure nearly all patients with 8-12 weeks of treatment. The treatments are oral and better tolerated than PR. PR treatment required weekly intravenous dosing, lasted 24-48 weeks, and cured ~50% of the patients treated.1,2 An interferon-free treatment era is thus around the corner. Significant efforts are now being made to optimize treatments with DAAs so that cure can be achieved with the least drug exposure and in the shortest possible time.3-8 Interferon, we believe, may have a new role here.
Interferon is a central player in our innate immune system.\(^9,^{10}\) It is a cytokine produced by cells in response to viral infections. Following its secretion, it binds cell surface receptors in a paracrine and autocrine manner and triggers a complex series of signaling events involving the JAK-STAT pathway. As a result, several hundred genes, termed interferon-stimulated genes (ISGs), are expressed, which collectively create an antiviral state in cells. Viral replication gets controlled in infected cells. Furthermore, neighboring cells acquire a heightened degree of protection, limiting the spread of the infection. HCV as well as other viruses have evolved sophisticated mechanisms to subvert the interferon response. For instance, HCV can suppress interferon production, interfere with the JAK-STAT pathway, block the translation of ISG mRNA, and inhibit the action of ISGs.\(^{10,11}\) A battle between interferon and HCV is thus fought in each cell. The outcome of these millions of micro battles is a key determinant of whether HCV can establish lasting, productive infection in individual target cells, and hence in the host. A strong endogenous interferon response may tilt the competition in favor of the host. Specifically, it may ease the burden on DAAs and improve the chances of cure even with interferon-free treatment regimens. Clinical data, which we summarize in this review, present evidence in support of this hypothesis. Patients with strong endogenous interferon responses may thus present a promising subpopulation for which the duration of DAA treatments can be reduced. Patients with weak endogenous interferon responses may benefit from adding interferon to their treatments.

To leverage interferon for optimizing DAA treatments, a quantitative understanding of its role in controlling HCV infection is necessary. The challenge here arises from the diverse length and time scales over which the action of interferon is manifested. At the cellular level, interferon acts as a signaling molecule triggering ISG expression and preventing viral replication and productive infection of target cells. At the level of the infected individual, the protection conferred by interferon, together with other factors such as the strength of the adaptive immune response, defines the severity of infection and the chances of cure. At the population level, the manifestation is in the success rate of different treatments. Independent experiments and clinical trials have characterized the intracellular interactions between interferon and HCV, measured viral load changes in individuals undergoing treatment with PR and with DAAs, and documented cure rates achieved in different populations with different treatment regimens. Experiments that establish quantitative links from the cellular to the population level and synthesize the above observations are difficult to conceive and perform. In recent studies, mathematical models have been constructed that quantitatively describe all of the above experimental observations and facilitate the establishment of these links.\(^{12,13}\) Here, we review the conceptual advances made by these models, highlight the insights they provide, and examine their implications and prospects for optimizing DAA treatments.

The rest of the review is organized as follows. In the next section, we summarize clinical evidence in support of interferon improving the response to DAAs. In the third section, we present an overview of the modeling framework that establishes links between the roles of interferon from the cellular to the population level. In Sections 4, 5, and 6, we describe the components of the framework at the cellular, individual, and population level, respectively, and examine their key predictions. At each stage, we draw links with existing models wherever possible and highlight similarities and differences. We end in Section 7 with an outlook on the key findings and their implications and prospects.

## 2 | Clinical Evidence That Interferon Can Improve DAA Treatment Outcomes

Does a better endogenous interferon response translate to better DAA treatment outcomes? To answer this question, a recent study\(^1\) considered all clinical trials involving DAAs and collated those DAA combinations and treatment regimens for which cure rates in both treatment-naive individuals and previous null responders to PR were reported. Null responders to PR are those who experience <2 log\(_{10}\) viral load decline in 12 weeks of therapy. Cure is assumed when treatment elicits a sustained virologic response (SVR), defined as undetectable plasma viremia 12 weeks after the end of treatment. The strength of the endogenous interferon response is difficult to measure, especially because the dynamics in the liver may be distinct from that in the blood.\(^14\) Individuals who previously failed PR treatment are expected to be less responsive to interferon than typical treatment-naive individuals. If interferon were to improve DAA treatment outcomes, SVR rates in a previous null responder population would be lower than in a treatment-naive population subjected to the same treatment regimen. From data of over 50 clinical trials,\(^15-65\) involving numerous single, pairs, and three drug combinations of about a dozen DAAs, administered with and without interferon, SVR rates in treatment-naive patient populations were found to be significantly higher than in previous null responders to PR (Figure 1; \(P \approx 10^{-59}\) overall using the chi-squared test). The difference remained significant when interferon-free regimens alone were considered (\(P = 0.007\)) and was starker when interferon formed part of the treatment (\(P = 10^{-65}\)). Patients with liver cirrhosis are considered difficult to treat. The difference was significant when cirrhotic patients alone were considered (\(P = 10^{-5}\)) and remained so with noncirrhotic patients (\(P = 10^{-29}\)). The difference diminished when powerful DAA combinations were used that elicited nearly 100% SVR. Nonetheless, the clinical evidence in support of the hypothesis that interferon can improve DAA treatment outcomes is overwhelming.

Two questions follow: (1) Can the role of interferon in improving DAA treatments be described mechanistically and quantified? This question assumes significance because interferon and DAAs have been thought to act independently; interferon upregulates the innate immune response in a generic manner,\(^15\) whereas DAAs target specific HCV proteins.\(^66\) Ribavirin has been argued to potentiate the activity of interferon.\(^67,68\) In vitro experiments have seen synergy between interferon and some DAAs,\(^69,70\) but the origins of the synergy remain to be fully established.\(^71\) (2) Can the role of interferon be leveraged to personalize and optimize DAA treatments? These...
questions form the subject of recent mathematical modeling studies, which we describe next.

3 | OVERVIEW OF THE MATHEMATICAL MODELING FRAMEWORK

The models considered the role of interferon at each of the underlying scales, from the cellular to the population level, in independent parts and devised novel ways of integrating them (Figure 2). At each step, quantitative contact was made with experiments. First, a comprehensive model of the intracellular interaction between the interferon signaling network and HCV was constructed to describe the fates of cells infected with HCV in response to stimulation with interferon. The network was found to exhibit bistability, with one steady state where the virus subverted the interferon response and thrived and the second where it was cleared by interferon. Cells in which the former fate was realized predominantly were refractory to interferon. Cells in which the latter fate was predominant were responsive to interferon. Signatures of the underlying bistability were evident in experiments, which the model quantitatively described. The fraction of cells in an individual that was responsive to...
interferon quantified the extent to which interferon could suppress infection in the individual. Next, standard models of viral kinetics were advanced by incorporating the distinct subpopulations of cells, refractory and responsive to interferon, to elucidate the role of interferon in inducing viral load changes during treatment. The resulting model quantitatively described all the patterns of viral load changes observed in patients treated with PR and explained several confounding observations associated with PR treatment.

Third, the model was extended to incorporate the influence of DAAs. DAAs directly suppressed viral replication, controlling the infection, but were susceptible to failure via viral mutation-driven development of drug resistance. Interferon was hypothesized to improve DAA treatment by restricting the replication space required for the development of drug resistance. The larger was the fraction of cells responsive to interferon, the smaller was the chance of the emergence of resistance to DAAs. The model thus predicted how an individual with a given degree of responsiveness to interferon would respond to DAAs.

Finally, a distribution of the degree of responsiveness of interferon across individuals in a population was posited in order to predict the fraction of individuals that would respond to a given DAA treatment regimen. The distribution was quantified by comparisons of model predictions with measurements of the distribution of baseline viral load and of SVR rates observed in a minimal set of clinical trials. The model then captured the differences in the SVR rates in treatment-naive individuals and previous null responders to PR observed above quantitatively, presenting an understanding of the role of interferon in improving DAA treatments. Furthermore, the model estimated the extent of increase in interferon responsiveness that could be achieved in an individual by adding interferon at standard dosages to the treatment. A new handle to systematically reduce treatment durations in a potentially personalized manner emerges.

Below, we describe the individual modeling components and their implications.

4 | INTERFERON AT THE CELLULAR LEVEL

4.1 | The problem of the response to interferon

Interferon signaling via the JAK-STAT pathway leading to ISG expression is well studied. Of the many hundred ISGs, those that exert a significant antiviral effect against HCV have been identified.

![Cellular level](image1.png)

**FIGURE 2** Schematic of the overall modeling framework. At the cellular level, the interferon signaling network in the presence of hepatitis C virus (HCV) is characterized by a double negative feedback motif, which yields bistability. HCV thrives in one steady state and is cleared in the other. Depending on the strength of the interferon response relative to the strength of its subversion by HCV, cells could admit the first steady state alone, both the steady states or the second steady state alone. They are accordingly termed interferon refractory (blue), bistable (yellow), and interferon responsive (green). At the level of the infected individual, the relative prevalence of these cellular phenotypes defines the outcomes of therapy. Interferon refractory cells continue to get infected and produce virions during therapy with pegylated interferon and ribavirin (PR). Uninfected bistable cells are protected from infection, but infected ones continue viral production. Interferon responsive cells are cured and protected. When the fraction of interferon refractory cells is low, treatment with direct-acting antiviral agents (DAAs) succeeds as both the wildtype and resistance-associated variants (RAVs) are controlled, whereas when the fraction is high, RAVs rise and induce treatment failure. At the population level, a distribution of the latter fraction exists. Individuals with the fraction smaller than a critical fraction (brown region) succeed. The critical fraction increases as more drugs are used in combination, improving sustained virologic response (SVR) rates.
The levels of their expression in response to stimulation with interferon as well as their effect in blocking HCV replication in infected cells have been quantified. At the same time, the mechanisms used by HCV in subverting the action of ISGs have been elucidated. Chief among them is the translational block induced by HCV via the dimerization and phosphorylation of protein kinase R (PKR) (Figure 3A).

The HCV genome is a positive-strand RNA molecule, which in infected cells acts as a template to produce negative-strand RNA. The negative-strand RNA typically exists complexed with its positive strand counterpart as double-stranded RNA (dsRNA). The negative-strand RNA in turn acts as a template to produce more positive-strand RNA. An infected cell can accumulate many tens to hundreds of positive-strand RNA genomes, which can be packaged and released as progeny virions. In the presence of dsRNA, the enzyme PKR is dimerized and autophosphorylated. Phosphorylated PKR phosphorylates the eukaryotic translation initiation factor 2α-GDP (eIF2α-GDP) and prevents...

**FIGURE 3** Interferon signaling in infected cells. (A) A schematic of the interferon signaling network in the presence of hepatitis C virus (HCV) demonstrating ISG production and HCV-induced translational block via protein kinase R (PKR) (left), which together yield a double negative feedback loop (right). The network includes ISG expression following stimulation of the JAK-STAT pathway with interferon and the resulting control of HCV replication by key ISGs. At the same time, it considers the suppression of ISG translation by HCV via PKR dimerization and autophosphorylation, and the resulting depletion of eIF2α-GTP due to the phosphorylation of eIF2α-GDP and the sequestration of eIF2B. These competing interactions between HCV and interferon give rise to the double negative feedback loop. (B) Model predictions of the steady state expression of HCV RNA levels for fixed ISG levels (blue) and ISG levels for fixed HCV RNA levels (red). The intersections of the curves yield the steady states of the network. The filled circles are stable and the empty circle is unstable. (C) HCV RNA levels measured 20 hours postinterferon exposure as a function of the time of interferon addition postinfection (green) and the corresponding model predictions (purple) without (top) and with PKR silencing (bottom). Note that the switch in the HCV RNA levels is not sharp because the data are averaged across cells and also because the steady states may not be achieved within 20 hours. (D) The steady state HCV RNA levels admitted by the system as ISG-induced control of HCV is repressed by the factor ω. Regions I, II, and III define cells that are interferon refractory, bistable, and interferon responsive, respectively.
alpha protein translation is less sensitive to eIF2alpha becomes depleted of eIF2alpha sequesters eIF2B and restricts its activity. Consequently, the cell failure. At the same time, with unfavorable alleles, a 25% chance ex-

Even with favorable alleles, there was a 20% chance of treatment
differences. A significant portion, however, remained unexplained.

those who responded.100-102 Furthermore, exposure to interferon as

to be higher in individuals who eventually failed treatment than in
those who responded.100-102 Furthermore, exposure to interferon as
part of treatment did not lead to a significant increase in ISG expres-
sion in the former individuals but did in the latter.100-102 The question
then arises: when does PR succeed and when does it fail? One possibility
is that differences in host genetics render individuals more or less re-
sponsive to PR. Indeed, whole-genome analyses to define correlates
treatment outcome identified single-nucleotide polymorphisms in
the interferon lambda gene locus as strong predictors of treatment
response.97,98 SVR rates could be as high as 80% in individuals with
favorable alleles and as low as 25% in individuals with unfavorable
alleles at this locus.99 A significant portion of the differences in the
responses of patients to PR was thus attributable to host genetic
differences. A significant portion, however, remained unexplained.
Even with favorable alleles, there was a 20% chance of treatment
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ting the interferon response by blocking ISG translation. The

ISGs in the absence of the translational block.72,77,81 The model
and the level to which HCV replication was diminished by each of
the ISGs in the absence of the translational block.72,77,81 The model
was then solved for its steady states.

The model displayed bistability (Figure 3B). In one stable steady
state, HCV levels were high and ISG protein levels were low. This
was the interferon refractory state. In the second stable steady
state, ISG protein levels were high and HCV was cleared. This was
the interferon-responsive state. Separating the two was an unstable
steady state of intermediate ISG and HCV levels. The stability of a
steady state is defined classically by its response to perturbations.
Perturbations from an unstable state are spontaneously amplified
and drive the system away from the steady state, whereas pertur-
bations from a stable state die down. The system would thus reside
in one of the two stable steady states, the chosen one depending on
the initial conditions. If the initial conditions were such that a cell had
significantly higher virus and/or lower ISG levels than those corre-
sponding to the unstable intermediate state, the cell would eventu-
ally reach the interferon refractory steady state. Otherwise, it would
be cleared of the infection.

Signatures of this bistable behavior were evident in in vitro inter-
feron time-of-addition experiments (Figure 3C).72 Here, cells were in-
fected with HCV and after a certain amount of time exposed to a fixed
concentration of interferon. Several hours later, the level of HCV in the
culture was measured. As the time when interferon was added was in-
creased, a switch from low- to high-eventual viral levels was observed.
This switch arises from the underlying bistability. When interferon is
added before the HCV level in cells crosses the unstable boundary,
interferon would eventually clear the infection. When it is added after,
however, HCV would thrive and reach the high viral level correspond-
ing to the refractory state. The model quantitatively captured these
observations. As further proof, when the experiments were repeated
with PKR-silenced cells, the switch was lost. Viral levels remained low
regardless of the time of interferon addition, indicating that HCV could
no longer subvert the interferon response. The bistability was elim-
inated and the system could only access the interferon-responsive
state, as predicted by the model.

4.2 A systems view of the interferon signaling network

Earlier studies identified the many molecular players and the mecha-
nisms involved in interferon-mediated control of HCV76-80 and the
subversion of these mechanisms by HCV,92,11 unravelling the many
fronts of the battle between HCV and interferon. The individual
fronts, however, were inadequate to predict the outcome of the bat-
tle. The key insight of the recent study was that the outcome of the
battle was not the result of these individual molecular interactions
between HCV and interferon but rather a systems-level, emergent
property of the interferon signaling network.12 The model identified
the underlying motif in the intracellular interferon signaling net-
work in the presence of HCV as a double negative feedback loop
(Figure 3A); Interferon prevented HCV replication via ISGs and HCV
suppressed the interferon response by blocking ISG translation. The
motif is expected to yield bistability,103 that is, the presence of two
stable steady states, representing two distinct outcomes of the com-
petition between interferon and HCV. To ascertain this, the model
built a comprehensive description of intracellular HCV replication,
interferon signaling and ISG expression, PKR dimerization and
phosphorylation, and its blockade of ISG translation (the number of
equations is too large to reproduce here). The underlying parameters
were estimated by comparisons of the predictions of the different
parts of the model with corresponding experiments. The parts were
thus designed to describe quantitatively the growth of positive- and
negative-strand RNA levels in cells in the absence of interferon
signaling,72,89 the level of expression of the key anti-HCV ISGs in
response to stimulation with interferon in the absence of HCV,104-107
and the level to which HCV replication was diminished by each of
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inated and the system could only access the interferon-responsive
state, as predicted by the model.
4.3 | Implications of bistability in the interferon signaling network

The insights above had several implications. First, the model predicted that cells in an infected individual would exhibit three distinct interferon response phenotypes (Figure 3D). Variations across cells within an individual in interferon receptor or other ISG expression levels or in host factors required for HCV replication would lead to different strengths of the interferon response relative to the strength of the translational block induced by HCV.\textsuperscript{108,109} Cells with strong ISG expression and effectiveness in controlling HCV would admit the interferon-responsive state alone. Cells with weak ISG effectiveness, in contrast, would admit the interferon refractory state alone. Cells with moderate ISG effectiveness would admit both states but realize one based on the initial conditions. The responsiveness of an individual to PR would thus depend on the fraction of cells refractory to interferon. The larger is the latter fraction, the poorer the response is likely to be. Models of viral kinetics may describe viral load changes during therapy more accurately if the distinct cellular phenotypes are accounted for (see below).

Second, the model presents a plausible explanation of the confounding observation of high pretreatment ISG expression in individuals who respond poorly to PR. The endogenous interferon response of an individual is the result of a combination of the level of interferon production and the strength of ISG effector function. Consider a cell that admits the interferon-responsive steady state alone. Before exposure to interferon, such a cell is expected to have low ISG mRNA levels and high viral levels. When interferon is added, its ISG levels rise and the virus is cleared. In contrast, consider a cell that admits the interferon refractory state alone. Following exposure to interferon, the ISG mRNA levels rise but are unable to clear the virus because of the strong translational block. Any further interferon addition is unlikely to alter this state. An individual with weak endogenous interferon production\textsuperscript{110,111} but with a preponderance of cells responsive to interferon is thus typical of those who respond to PR. The individual displays low ISG levels pretreatment, a rise in ISG levels following the start of treatment, and an eventual response to PR. An individual with strong endogenous interferon production but with a preponderance of cells of the interferon refractory phenotype is representative of nonresponders to PR. Such an individual displays high ISG levels pretreatment, no change following the start of treatment, and suffers a failure of treatment. Individuals with moderate interferon synthesis levels and intermediate levels of interferon refractory and responsive cells are likely to be partial responders to PR (see below). Finally, individuals with high endogenous interferon synthesis levels and high levels of interferon-responsive cells are likely to clear the infection spontaneously.

Third, the model unraveled a new mechanism of synergy between DAs and PR. DAs target specific HCV proteins and reduce viral replication. An expected consequence is the decrease of viral RNA in infected cells. A second consequence is predicted by the model. Lower viral RNA levels diminish the strength of the HCV-mediated block of ISG translation. ISGs then exert greater control over HCV and can potentially convert an interferon refractory cell into an interferon-responsive cell. DAs can thus alter the systems level properties of the interferon signaling network and synergize with interferon in effecting viral control. Synergy between DAs and interferon has been observed in vitro\textsuperscript{69,70} and in vivo.\textsuperscript{112-115} Whether the mechanism of synergy underlying the observations is the one suggested here remains to be ascertained.

Nonetheless, the model suggested a novel approach to describing within-host viral kinetics, which we review next.

5 | INTERFERON AT THE INFECTED INDIVIDUAL LEVEL

5.1 | A bird’s-eye view

Modeling viral kinetics within infected individuals has had a rich history.\textsuperscript{116,117} This year marks the 20th year since the seminal paper by Neumann et al was published, where the first model of HCV kinetics was constructed, inspired by the success of similar models of HIV dynamics, and applied to analyze data from patients undergoing treatment with interferon.\textsuperscript{73} The model abstracted the underlying kinetics into the time evolution of three interdependent populations, namely, of target cells, infected cells, and free virions, within an infected individual. The populations evolved according to the following equations.

\[
\frac{d{s}}{dt} = s - d_{T} (1 - \eta) V_{T} - \delta T
\]

\[
\frac{d{T}}{dt} = (1 - \eta) k V_{T} - \delta I
\]

\[
\frac{d{V}}{dt} = (1 - \epsilon) p I - c V
\]

Here, free virions, $V$, infected target cells, $T$, with a second-order rate constant $\eta$, to produce infected cells, $I$, which in turn produced more virions at the per capita rate $\rho$. Infected cells and free virions were lost with fixed half-lives, determined by the per capita rates $\delta$ and $c$, respectively. Target cells were produced at the rate $s$ and lost with the per capita rate $d_{T}$. Interferon was hypothesized to prevent de novo infection with effectiveness $\eta$ and block viral production from infected cells with effectiveness $\epsilon$. During the short period following the start of therapy, for which data were analyzed, the target cell population was assumed not to change significantly. Before treatment, viral production and clearance were balanced ($pI_{s} = cV_{s}$), and so were infected cell production and loss ($\rho V_{ss} = \delta V_{ss}$), yielding a steady viral load, $V_{ss}$, often referred to as the baseline or set point viral load. (The subscript ‘ss’ refers to steady-state quantities before the start of treatment.) Treatment with interferon upset this balance and induced a biphasic decline in viral load. The first phase was attributed to the imbalance between viral production and clearance, which was fast, and lasted 1-2 days, at which point, the viral load reduced approximately to $V_{ss}(1 - \epsilon)$. The second phase was due to the resulting imbalance between the production and loss of infected cells, was slower, and typically lasted the rest of the treatment duration (many weeks),...
where the viral load declined with a slope approximately equal to \( \delta e \). The model fit patient data of biphasic viral load decline quantitatively, yielded estimates of the virion and infected cell half-lives, argued that interferon had little effectiveness in blocking de novo infection of cells, and identified a critical effectiveness of interferon in blocking viral production, \( \epsilon c \), for the treatment to succeed.

Significant advances have been made to the model over the years, each advance addressing a new puzzle and bringing us closer to a comprehensive description of HCV kinetics and treatment response. For instance, the model was advanced to incorporate drug pharmacokinetics, which became important with the once weekly dosing of pegylated interferon where drug concentrations showed significant variations between doses.\(^{118,119}\) The role of ribavirin in improving SVR in combination with interferon was described assuming that ribavirin rendered virions noninfectious.\(^{67}\) Models that suggested optimal ribavirin usage, which kept its key side effect, hemolytic anemia, tolerable, were also constructed.\(^{120,121}\) Homeostatic proliferation of hepatocytes was incorporated to explain the triphasic decline of viremia observed in some patients.\(^{122}\) With the advent of DAAs, viral mutation and the development of drug resistance became important and were incorporated to describe the kinetics of resistant strains and to predict the minimum genetic barrier of DAA combinations required to prevent treatment failure.\(^{123}\) More recently, multiscale models that couple intracellular viral replication with within-host kinetics have been constructed to describe the rapid viral load decline observed with some of the new DAAs.\(^{124}\) Finally, models are now being constructed that explicitly incorporate the influence of the adaptive immune system to describe the intriguing recent observation of patients achieving SVR despite viremia being detected at the end of treatment with DAAs.\(^{125-127}\) Several excellent reviews have documented these and other advances and highlighted their contributions to our understanding of HCV pathogenesis and the design of improved treatment protocols.\(^{116,117}\) Here, we focus on a recent model\(^ {12,13}\) that advances the basic model by incorporating the distinct cellular interferon response phenotypes described above and enables a more accurate description of the influence of interferon on HCV kinetics.

### 5.2 Viral kinetics with distinct cellular phenotypic responses to interferon

Based on the implications of the bistability of the intracellular interferon signaling network elucidated above, the model divides the hepatocyte population in an infected individual into three subpopulations (Figure 4A).\(^ {12}\) The first comprises those that admit the interferon refractory steady-state alone. These cells are unaffected by PR blocks productive infection of target cells with effectiveness \( \eta \) and inhibits viral production from infected cells with effectiveness \( \epsilon \). The response phenotypes imply that \( \eta_1 = \epsilon_1 = \epsilon_2 = 0 \) and \( \eta_2 = \eta_3 = \epsilon_3 = 1 \). (B) Fits of model predictions (lines) to data of viral load changes (symbols) during PR therapy in a responder (blue), partial responder (red), and null responder (green). The initial viral load, \( V_{ss} \), \( \beta \), and \( \phi_1 \) were adjustable. Poorer responses yielded higher values of \( \phi_1 \).

**FIGURE 4** Viral kinetics during pegylated interferon and ribavirin (PR) therapy. (A) A schematic illustrating the extension of the basic model to account for the three cellular interferon response phenotypes. Subscripts 1, 2, and 3 represent the interferon refractory, bistable, and interferon responsive phenotypes, respectively. The phenotypes arise due to the different relative strengths of interferon-mediated control of hepatitis C virus (HCV) replication and the subversion of the interferon response by HCV. These are shown inside infected cells symbolically as strong (solid line) and weak (dashed line). In each category, target cells \( T \) are produced at rate \( s \), die at rate \( d \), and are infected by virions \( V \) at the rate \( \beta \) to yield infected cells \( I \), which proliferate at the rate \( r_I \), die at the rate \( \delta \), and produce virions at the rate \( p \). Free virions are cleared at the rate \( c \). (The rates are all per capita or represent suitable rate constants.) The fractions of cells in the different phenotypes \( \phi_1, \phi_2, \phi_3 \) determine \( \phi_2 = s \phi_3 \) and so on. PR blocks productive infection of target cells with effectiveness \( \eta \) and inhibits viral production from infected cells with effectiveness \( \epsilon \). The response phenotypes imply that \( \eta_1 = \epsilon_1 = \epsilon_2 = 0 \) and \( \eta_2 = \eta_3 = \epsilon_3 = 1 \). (B) Fits of model predictions (lines) to data of viral load changes (symbols) during PR therapy in a responder (blue), partial responder (red), and null responder (green). The initial viral load, \( V_{ss} \), \( \beta \), and \( \phi_1 \) were adjustable. Poorer responses yielded higher values of \( \phi_1 \).
interferon. They get infected and produce progeny virions even in the presence of interferon. The second subpopulation comprises cells that admit both the interferon refractory and responsive steady states. At the start of treatment, if a cell in this subpopulation is infected, it is likely to have viral levels higher than that defined by the intermediate unstable steady state so that addition of interferon cannot prevent the cell from reaching the interferon refractory state. The cell thus continues to produce progeny virions. An uninfected cell in this subpopulation, however, gets exposed to interferon at the start of therapy before it can encounter a virion. The cell is thus driven to the interferon-responsive state and is protected from infection. The third subpopulation comprises cells that admit the interferon-responsive state alone. Infected cells in this category are cured once treatment with interferon is initiated. Uninfected cells in the category become immune to the virus. The following equations then describe the ensuing viral kinetics:

\[
d_{T\text{i}} = \frac{\phi_i + r_T}{T_i} \left[ 1 - \frac{d_T}{d_{\text{max}}} \right] - (1 - \eta_i) \delta V_{T\text{i}} - d_{T\text{i}}; \quad i \in \{1,2,3\}
\]

\[
d_{T\text{i}} = r_T \left[ 1 - \frac{d_T}{d_{\text{max}}} \right] + (1 - \eta_i) \delta V_{T\text{i}} - d_{T\text{i}}; \quad i \in \{1,2,3\}
\]

\[
d_{V\text{i}} = p \sum_{i=1}^{\text{3}} (1 - \epsilon_i) I_i - cV
\]

Here, the subscript \(i \in \{1,2,3\}\) distinguishes the target cells, \(T\), and infected cells, \(I\), in the three subpopulations above. \(\phi_i\) represents the fraction of target cells produced that are of type \(T\). Target cells and infected cells proliferate with per capita rates \(r_T\) and \(r_I\), respectively, tempered by the logistic term that restricts the maximum hepatocyte population to \(T_{\text{max}}\), including the subpopulation of cells that are not targets of infection, \(N\), due for instance to inadequate entry receptor expression. \(\delta v_{T\text{i}}\) interferon blocks de novo infection and viral production from the respective cell types with effectiveness \(\eta_i\) and \(\epsilon_i\). The remaining terms have the same meanings as in the basic model (Equation 1 above).

It follows from the description above of the response of the different cellular subtypes to interferon that \(\eta_2 = 0\) and \(\eta_3 = 3\), whereas \(\epsilon_1 = \epsilon_2 = 0\) and \(\epsilon_3 = 1\). Thus, the cells in the first subpopulation are the ones that sustain the infection. They provide new target cells that can be productively infected during interferon therapy. The infected cells in the second subpopulation produce virions until they die but are then not replenished and so do not influence long-term dynamics. The fraction of cells in the first subpopulation, determined by \(\phi_2\), thus defines the degree of responsiveness to interferon. The smaller is the fraction, the better is the response. Indeed, by varying \(\phi_2\), the model described all the patterns of viral kinetics observed in patients treated with PR\(^{73}\) (Figure 4B). When \(\phi_2\) was small, viral load declined rapidly, in a biphasic manner, and led to SVR. With higher \(\phi_2\), the extent to which viremia reduced was lowered, but the virus did not get cleared during treatment, indicating a partial response. With very high \(\phi_2\), hardly any decline in viremia was observed, marking a null response. The model provided excellent fits to data from patients experiencing each of these patterns of response.

An alternative interpretation of the two phases of viral load decline emerges. The first phase is defined by the size of the interferon-responsive cell subpopulation, which gets cured following the start of treatment and causes a steep decline in viremia. It can be shown that the viremia at the end of the first phase is \(V_{\text{C1}}(1 - \phi_2)\). The second phase slope is dictated by the loss of infected cells and depends on the size of the interferon refractory subpopulation. The larger the value of \(\phi_2\), the smaller the slope. The early second phase slope can be shown to be \(\delta(1 - \phi_2)\). Indeed, analyzing data from a large number of patients on interferon monotherapy, \(^{129,130}\) the second phase slope was found to be inversely correlated with \(\phi_2\). Furthermore, patients with single-nucleotide polymorphisms in the interferon lambda gene locus that correlate with better treatment response were found to have lower \(\phi_2\) than those with polymorphisms that implied poor response. \(^{131,132}\) Finally, a critical value of \(\phi_2\), denoted \(\phi_{\text{crt}}\), could be defined below which PR treatment would succeed and above which it would fail. The critical fraction is akin to the critical drug efficacy, \(\epsilon_c\), defined in previous models (see above) as necessary for successful treatment. \(^{122}\) The model thus recapitulated all the key predictions of the basic model but did so using a structure that is rooted in the properties of the interferon-signaling network.

Importantly, the model presented an avenue to describe the influence of interferon at the population level. The fraction of individuals with \(\phi_2\) below \(\phi_{\text{crt}}\) would yield the SVR rates observed with PR treatment. The problem of predicting SVR rates thus gets mapped to the potentially simpler problem of estimating the distribution of values of \(\phi_2\) across individuals.

\(\phi_2\) is not easy to estimate in individuals a priori. Whether a cell would be responsive or refractory to interferon is determined by many factors including its interferon receptor expression level, the concentrations of the players in the JAK-STAT pathway, the ISG expression levels, and the strength of the HCV-mediated interference of the interferon response. Variations in these factors across cells remain difficult to quantify. The distribution of \(\phi_2\) was estimated indirectly by extending the model to the population level and comparing predictions with observed SVR levels. We consider this extension next.

## 6 | INTERFERON AT THE POPULATION LEVEL

### 6.1 | A mechanistic hypothesis

The motivation for building a model of the response to interferon at the population level arose from the compelling clinical evidence, presented at the start of this review, of a superior response to DAAAs in treatment-naive patients than in previous null responders to interferon. Previous models have used empirical arguments to describe this differential response. \(^{133-135}\) A
mechanistic explanation was lacking. The model of interferon action at the individual level described above allowed the construction of a mechanistic hypothesis to explain this differential response. DAA failures primarily due to the development of drug resistance.74,75 Viral mutation forms the route through which resistant strains arise. HCV has high mutation88 and replication73 rates and exists in infected individuals as a quasispecies.123,136 Many mutations can give rise to resistance to individual DAA.137,138 Thus, the chance that resistant strains exist in infected individuals before the onset of treatment can be high.123 Previous models that coupled viral kinetics with viral mutation predicted that all single and double mutants are likely to preexist in infected individuals and an additional mutation is likely to arise during therapy, leading to the recommendation of a minimum genetic barrier of 4 for DAA combinations to succeed.123 Following these arguments, if DAA work better in individuals with stronger interferon responses, it is likely that interferon compromises the ability of the virus to develop resistance to DAA. A model that superimposes viral kinetics and evolution on the formalism above of the distinct cellular interferon response phenotypes would help test this hypothesis. Such a model was constructed.13

6.2 | Viral kinetics with interferon and DAA

The model again divided cells into the three interferon response phenotypes, with the pretreatment fraction of cells refractory to interferon, that is, the pretreatment $\phi_1$, denoted $\phi_p$ (Figure 5A). Cells could be infected with either the wildtype viral strain, denoted $V_0$, or a mutant, denoted $V_j$. Cells infected with the wildtype produced a majority of wildtype progeny virions and a small proportion of mutants. The proportion was dictated by the mutation rate, $\mu$. Cells infected with mutant strains would similarly produce a majority of mutant virions. The viral burst size and/or infectivity of the mutant strains was lower (ie, $p_0 > p_j$ and/or $\beta_0 > \beta_j$), representing the fitness cost associated with the resistance mutation.137 The resulting model equations were as follows.

\[
\frac{dV_0}{dt} = \phi_p + \epsilon T \left[ 1 - \frac{\sum_{i=1}^{3} (1-r_j i) \beta_i}{\sum_{i=1}^{3} (1-r_j i)} \right] - \frac{(1-\eta)}{\sum_{i=1}^{3} (1-r_j i)} \beta V_j - \frac{dT}{dt}; \ i \in \{1,2,3\}
\]

\[
\frac{dV_j}{dt} = -\epsilon T \left[ 1 - \frac{\sum_{i=1}^{3} (1-r_j i) \beta_i}{\sum_{i=1}^{3} (1-r_j i)} \right] + \frac{(1-\eta)}{\sum_{i=1}^{3} (1-r_j i)} \beta V_j - \frac{dT}{dt}; \ i \in \{1,2,3\}, j \in \{0,1\}
\]

\[
\frac{dv_0}{dt} = (1-\mu)(1-\epsilon_0 DAA) \phi_0 \sum_{i=1}^{3} (1-r_i) \beta V_j - cV_0
\]

\[
\frac{dv_j}{dt} = \mu(1-\epsilon_0 DAA) \phi_0 \sum_{i=1}^{3} (1-r_i) \beta V_j + (1-\epsilon_1 DAA) \phi_1 \sum_{i=1}^{3} (1-r_i) \beta V_j - cV_1
\]

Here, in addition to distinguishing cell types based on their interferon response phenotypes, $i \in \{1,2,3\}$, cells are distinguished by the viral types that infect them, viz., sensitive and resistant to the DAA involved, denoted using $j \in \{0,1\}$ (The other notations and terms are similar to those in Equation (2) above). The model predicted, interestingly, that the pretreatment frequency of the mutant was independent of $\phi_p$. This was because as $\phi_p$ increased, the infected cell population increased, allowing greater viral replication, but it did not alter the distribution of virions produced from each cell. Thus, the frequency of mutants was unaffected. In absolute numbers, however, the viral and infected cell populations increased with $\phi_p$. During treatment, the higher viral and infected cell populations resulted in an increased chance of the development of resistance and treatment failure. The model defined $\phi_1$ as the fraction of cells refractory to interferon during treatment, that is, $\phi_1$ was the value of $\phi_p$ during treatment. If interferon was not part of the treatment, $\phi_1$ was equal to $\phi_p$. Else, $\phi_1$ was less than $\phi_p$ because exogenous interferon would increase net interferon levels in circulation and improve responsiveness. DAA worked with different effectiveness, denoted $\epsilon_0 DAA$ and $\epsilon_1 DAA$, against the wildtype and the mutant strains, respectively.137 The model predicted that given a set of drug effectiveness values,
a critical value of $\phi_t$ existed below which the treatment succeeded. The critical value was akin to the critical fraction $\phi^{\text{critical}}$ identified above in the absence of DAAs. Thus, greater interferon responsiveness (lower $\phi_t$) resulted in improved DAA treatment outcomes.

### 6.3 The distribution of interferon responsiveness and the success rates of treatments

To examine whether this prediction translated to the SVR rates observed, the following approach was used. $\phi_p$ was assumed to be distributed log normally across individuals in a population. The pretreatment steady state of the model showed that $\phi_p$ was directly linked to the baseline viral load: $\phi_p = \frac{V_{\text{ss}}}{V_{\text{max}}}$, where $V_{\text{max}}$ was the maximum pretreatment viral load, achieved when all cells are interferon refractive, that is, $\phi_p = 1$. From the known distribution of the baseline viral load, thus, parameters defining the distribution of $\phi_p$ were estimated. With this distribution, the model was able to describe several previously unexplained clinical observations.

The model yielded a nonzero baseline viral load only when $\phi_p > \phi_c$. Individuals with $\phi_p < \phi_c$ thus represented those who spontaneously cleared the infection (Figure 5B). From the distribution of $\phi_p$, the percentage of spontaneous clearers was estimated to be ~21%, which was close to the mean of ~26% obtained from 31 longitudinal studies.
The distribution of $\phi_p$ truncated below by $\phi_c$ yielded the distribution of $\phi_p$ in chronically infected treatment-naive individuals (Figure 5C). To compare the response elicited in this population with that in null responders to PR, the distribution of $\phi_p$ in null responders was required.

Null response to PR was defined to occur when $\phi_p > \phi_{null}$. To estimate $\phi_{null}$, the extent to which interferon increases responsiveness, $\Delta\phi = \phi_p - \phi_c$, had to be determined. The following argument was used. Consider a DAA which when administered alone results in SVR when $\phi_p < \phi_{DAA}$. When the DAA is used in combination with PR, let SVR be achieved when $\phi_p < \phi_{PR+DAA}$. Clearly, $\phi_{PR+DAA} > \phi_{DAA}$ as individuals with weaker interferon responsiveness can achieve SVR when interferon is added exogenously. Recall that SVR is achieved when the viral load reaches the cure boundary of one virion in the 15 liters of extracellular fluid in a typical individual. The threshold $\phi_{DAA}$ is that value of $\phi_p$ for which the cure boundary is reached exactly at the end of treatment with the DAA. A higher $\phi_p$ would imply the presence of the virus at the end of treatment, which would reignite infection subsequently and cause treatment failure (see caveat below). A lower $\phi_p$ would imply complete viral clearance before the end of treatment, signifying suboptimal treatment. Similarly, $\phi_{PR+DAA}$ is that value of $\phi_p$ for which the cure boundary is reached exactly at the end of treatment with the PR+DAA combination. (We note here that an alternative cure boundary of 1 infected hepatocyte in the body has been proposed, which typically requires longer treatment durations to achieve. For a given treatment duration, the latter cure boundary would result in lower estimates of $\phi_{DAA}$ and $\phi_{PR+DAA}$. Whether these estimates are consistent with clinical data remains to be examined). The two critical scenarios, namely $\phi_p = \phi_{DAA}$ with the DAA alone and $\phi_p = \phi_{PR+DAA}$ with the PR+DAA combination, yielding identical kinetics is possible when $\phi_c$ which defines the kinetics for nearly the entire period during treatment excepting the initial transients, corresponding to the two scenarios is the same. The cure boundary is then reached at the same time in both the scenarios. As $\phi_p = \phi_c$ with the DAA alone and $\phi_c = \phi_p - \Delta\phi$ with the PR+DAA combination, it follows that $\Delta\phi = \phi_{PR+DAA} - \phi_{DAA}$.

Data for telaprevir, one of the early DAAs, was used to apply the above argument and estimate $\Delta\phi$. The model of viral kinetics was used with the known efficacies of telaprevir against the wildtype and mutant strains and the relative fitness of the mutant to estimate $\phi_{DAA}$. For this, the model was solved for different values of $\phi_p = \phi_1$ and the value that drove viremia to the cure boundary just at the end of treatment was identified. Clinical data of SVR rates with the telaprevir and PR combination were known. From the population distribution of $\phi_p$, this SVR rate yielded $\phi_{PR+DAA}$ as that value of $\phi_p$ below which the percentage of individuals in the population was equal to the SVR rate. The difference provided an estimate of $\Delta\phi$.

The combination of the individual and population level models thus yielded, remarkably, an estimate of the degree to which interferon responsiveness is increased by PR within treated individuals.

If the contribution from the synergy between telaprevir and PR was small, the increase in responsiveness was attributable to PR alone and would hold for PR treatment and for the cases where PR is used with other DAAs and the synergy is similarly small. In particular, the estimate of $\Delta\phi$ helped define $\phi_{null}$. Using the viral kinetic model for PR treatment, with $\Delta\phi$ now quantified, the study estimated $\phi_{null}$ as the value of $\phi_p$ for which $2\log_{10}$ decline in the viral load occurred exactly in 12 weeks of PR therapy. Using this value with the distribution of $\phi_p$ yielded the percentage of null responders to PR to be 33%, in remarkably close agreement with the clinically observed 32%.

The distribution of $\phi_p$ truncated below by $\phi_{null}$ yielded the distribution of $\phi_p$ in null responders (Figure 5D). SVR rates in null responders could thus be predicted. Indeed, using the value of $\phi_{PR+DAA}$ above, the SVR rate elicited by the combination of telaprevir and PR in null responders to PR was estimated to be 26%, again in good agreement with the 32% observed clinically.

The model now had all the ingredients required to predict SVR rates elicited by any combination of DAAs with or without PR in a treatment-naive population as well as in a population of previous null responders to PR given the effectiveness of the drugs and the costs of resistance mutations. Spanning a range of values of these parameters, potentially encompassing all drug combinations, model predictions captured the clinical data summarized earlier quantitatively (Figure 1B). Remarkably, the SVR rates elicited in null responders varied linearly with the SVR rates in treatment-naive populations. The study showed how this linear relationship could be understood through a simple analysis of the distribution of $\phi_p$ and the different threshold values of $\phi_p$ (Figure 5E). The analysis indicated further that the difference in the SVR rates between treatment-naive individuals and previous null responders to PR would vanish as the DAAs became efficacious and elicited 100% SVR, as observed with the more potent combinations today (Figure 1). The study thus presented a quantitative understanding of how responsiveness to interferon could improve the outcomes of DAA treatments including interferon-free combinations.

The implications for optimizing DAA treatments could be significant. Individuals with higher interferon responsiveness are predicted to achieve SVR faster than those with weaker interferon responsiveness even with interferon-free DAA combinations. The minimum treatment duration for achieving SVR can be estimated as a function of $\phi_p$ given the effectiveness of the DAA combination in question (Figure 5F). This prediction can provide inputs to response guided therapeutic strategies. The extent of viral load decline in the first phase presents an estimate of the fraction of cells responsive to interferon. The remaining fraction thus is an upper bound on the fraction of cells refractory to interferon. The required treatment duration may thus be estimated in a potentially personalized manner. Where the duration predicted may be large with DAAs alone, the addition of interferon may provide an additional option.

**OUTLOOK**

Overwhelming clinical evidence points to better responses to DAAs, including to interferon-free combinations, in patients with stronger endogenous interferon responses. Recent mathematical
models provide explanations of this intriguing correlation.\textsuperscript{12,13} The advance made by these models is the integration of the manifestation of the action of interferon at the cellular, individual, and the population level into a single mathematical framework. Consequently, the models are able to describe a large body of clinical observations quantitatively, including the percentage of infected individuals that spontaneously clears the infection, the percentage of chronically infected individuals that fails to respond to interferon, and the percentage of the latter that responds to DAAs. The models and the clinical evidence suggest that interferon could be leveraged to improve outcomes of the new DAA combination treatments. Individuals with strong interferon responsiveness could be a promising subpopulation for reducing treatment durations. The models suggest that short-term viral load decline could provide an estimate of the degree of interferon responsiveness of an individual. Future studies could establish correlations between the short-term viral load decline and the corresponding interferon responsiveness, which could then be exploited to estimate the minimum duration of treatment required to achieve SVR in a potentially personalized manner.

Despite their complexity, the models are restricted to descriptions of the essential roles of interferon. A more comprehensive description of the role of interferon would require addressing several additional issues. The models, for instance, do not present a way to estimate the interferon responsiveness of an individual a priori. DAAs and interferon have been shown to synergize via multiple mechanisms. Although the models identify a mechanism of such synergy, they neglect its effects in defining treatment outcomes. While the collated clinical data appear well described despite this simplifying approximation, specific cases where DAAs exhibit strong synergy with interferon may be inadequately captured. The possibility of synergy implies that the models yield conservative estimates of the required treatment duration. The treatment duration in the models is based on driving viremia below the cure boundary of one virion in the 15 l of fluid volume in a typical individual. Alternative descriptions of the cure boundary have been proposed—for instance, one infected cell in the body instead of one virion—and this can alter the predicted treatment duration.\textsuperscript{116} Whether the models can similarly be applied to describe clinical data with these alternative cure boundaries remains to be examined. Furthermore, more recent observations where some patients with detectable viremia at the end of treatment also achieved SVR suggest that the required treatment durations may be shorter than estimated based on the above cure boundaries.\textsuperscript{8,143-150} Indeed, in a recent study, noncirrhotic Chinese individuals infected with HCV genotype 1b who displayed an ultrarapid early response (viremia <500 copies/ml by day 2 of treatment) were found to achieve SVR with just 3 weeks of therapy.\textsuperscript{5} The mechanisms underlying the spontaneous achievement of SVR despite detectable viremia at the end of treatment remain to be elucidated. Some studies suggest that DAAs irreversibly diminish the infectivity of the virus to a point where not enough infectious virions are left at the end of treatment to establish a lasting infection.\textsuperscript{125,126} Other studies argue that the reduction in viremia due to DAAs could reverse the exhaustion of cytotoxic T lymphocytes,\textsuperscript{154-157} which then clear the infection. Mathematical models based on both these hypotheses appear to be consistent with patient data.\textsuperscript{125-127} Interferon has been argued to enhance exhaustion late in infection\textsuperscript{158-160} and is thus likely to influence outcomes differently depending on which mechanism predominates. Future studies that establish the underlying mechanism would further clarify the role interferon can play and help refine the predictions of the minimum treatment duration made with present models.

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**CONFLICT OF INTEREST**

The authors declare that they do not have any conflicts of interest.

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