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# The rapid viral decline with the HCV NS5A inhibitor daclatasvir reveals a dual mode of action and leads to a new HCV half-life estimate

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Chronic infection with hepatitis C virus (HCV) affects approximately 160 million people worldwide [1] and is the leading cause of cirrhosis, liver cancer and liver transplantation [2]. Until 2011, the standard of care was weekly injections of pegylated-interferon-α (peg-IFN) and daily oral ribavirin (RBV), but viral eradication was achieved in less than 50% in treatment naïve HCV genotype 1 patients, the most prevalent genotype in Western world [3].

Since the approval in 2011 of two protease inhibitors (PI), telaprevir and boceprevir, in combination with peg-IFN/RBV, the landscape of anti-HCV therapy has been rapidly changing. Dozens of direct antiviral agents (DAAs) targeting different stages of the viral lifecycle are currently in clinical development, holding the promise that a universal cure can be achieved. Although early results, often obtained in small populations of highly selected patients, should be taken with caution, short, tolerable, pan-genotype and IFN-free regimens appear today as being attainable within the next couple of years.

In this highly dynamical context, there has been an increasing appeal from industry and academics to use viral kinetic mathematical modeling to anticipate, evaluate and rationalize the effectiveness of these new antiviral strategies [4]. This is particularly true in the case of agents that target proteins with complex or ill-defined functions, such as the non-structural (NS) protein 5A. In a recent paper published in PNAS [5], we employed mathematical modeling to decipher the origin of the very rapid viral decline observed in the first hours following initiation of daclatasvir (DCV), a first-in-class NS5A inhibitor, where one dose of DCV led to a 1000-fold decrease in viral levels within ~12 hours [6].

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## Hepatitis C viral dynamics, virion half-life in serum and drug antiviral effectiveness

One of the most important results of viral kinetic modeling was to introduce the idea that viral load level observed in serum reflects the balance between the antagonist processes of production and clearance of virus. By blocking viral production from infected cells, the initiation of antiviral treatment disrupts this equilibrium and the clearance of virus is no longer efficiently compensated. Thus the rate of viral decline observed early after treatment initiation can be used to calculate the virus half-life in serum. This basic idea was used by Neumann and colleagues [7] to fit the viral decline observed during IFN therapy and to estimate the antiviral effectiveness of IFN as well as to obtain the original estimate of the HCV half-life of 2.7 hours. Importantly, if HCV is rapidly eliminated from serum it implies that large quantities of virus (about $10^{11}$–$10^{13}$ virions) must be produced every day to maintain viral levels of $10^{6}$–$10^{8}$ HCV RNA copies/mL in the absence of treatment. It is important to understand that the clearance rate of HCV reflects a physiological quantity that should not depend on the type of treatment. Thus in the framework of viral dynamic models, the initial rate of viral decline, which reflects HCV clearance, should be roughly similar across treatments. In fact a similar half-life was repeatedly found in subsequent studies, where different doses of IFN or peg-IFN in HCV genotype 1 were analyzed. Interestingly, an exception to this estimate of HCV half-life arose in a study involving 44 patients treated with telaprevir (TVR) [8], where a HCV half-life of 1.2 hours was estimated.

## HCV half-life in serum estimated during daclatasvir therapy

The analysis of the viral load kinetics with DCV revealed a much more striking difference, with all 9 patients receiving 10 or 100 mg of DCV having a roughly similar and very rapid rate of viral load decline, corresponding to a HCV half-life of about 45 minutes. Moreover, the viral load assessments were very frequent with 8 blood samples performed within the first 24 hours, allowing a very precise estimate of this parameter. Drug pharmacokinetics was also measured and more complex models that integrate the changes in drug effectiveness arrived at the same estimate (data not shown).

## Why do IFN and daclatasvir generate different estimates of the HCV half-life?

We hypothesized that this discrepancy may be due to different modes of action for the drugs. Indeed the standard viral kinetic model [7] disregards the possibility that the rapidity of viral decline may be contingent on the specific stages of the viral lifecycle that are affected by a given molecule. For instance blocking either the NS5B polymerase or viral secretion both result in blocking viral production. However, for a given level of effectiveness, the latter will result in an earlier viral decline than the former, since blocking viral replication will not prevent already existing intracellular viral RNA (vRNA) from being assembled and secreted, and thereby delaying or slowing the viral decline. As a general rule the later in the virus lifecycle a drug acts, the more rapid the decline should be. This led us to use a novel modeling approach that incorporates possible drug effects on the

HCV intracellular lifecycle, namely blocking assembly/secretion, vRNA replication or enhancing vRNA degradation, to understand the mechanisms that govern the early virologic response to treatment.

## Effectiveness of DCV in blocking two distinct stages of viral lifecycle

We used this new model to simultaneously fit the viral kinetics during standard high dose daily IFN, TVR monotherapy and after a single dose of DCV. Interestingly all patients, including those not receiving DCV, could be fitted assuming a HCV half-life of about 45 minutes. All drugs had a high effectiveness in blocking synthesis of new viral genomes but had significantly different levels of effectiveness in blocking virus assembly/secretion, with DCV blocking 99.7% of assembly/secretion versus 44% ($P<10^{-10}$) and 94% ($P<10^{-6}$) for IFN and TVR, respectively. Moreover we estimated the intracellular vRNA half-life to about 12 hours in patients treated with IFN and DCV versus 4.3 hours in patients treated with TVR. In a subsequent study, involving patients receiving danoprevir [9], another protease inhibitor, a similar result was found suggesting that protease inhibitors, unlike IFN or DCV, may have an effect in enhancing intracellular vRNA loss. This finding may be related to the effect of protease inhibitors in blocking NS3-mediated inhibition of host innate antiviral pathways.

As a consequence of its dual mode of action, DCV leads to the most rapid HCV RNA decline observed to date and provides a more precise estimate of the HCV half-life in serum. On the other hand, because IFN and to a lesser extent TVR, had a much lower effectiveness in blocking viral assembly/secretion the virus decline with these compounds was much slower than with DCV leading to the previous overestimates of the HCV half-life.

## Validating modeling predictions using viral kinetics in the infectious clone system

Although viral kinetic modeling predicts that DCV acts to prevent both vRNA replication and viral/assembly secretion, it is important to validate these predictions before accepting them. To do this we established a collaboration with Susan Uprichard, an experimental virologist at Loyola University Chicago. Using the JFH-1 infectious clone system [10,11], Uprichard and her PhD student, Natasha Sansone, were able to infect non-growing Huh-7 cells with HCV [12], allow the culture to attain steady state and then treat with either a nucleoside-polymerase inhibitor, NM107, or DCV at doses that gave equivalent declines in intracellular vRNA. However, when extracellular virus was titered, there was a striking difference between cultures treated with NM107 and DCV. As one would expect of a replication inhibitor, such as NM107, extracellular viral titer declined in parallel with the decline in intracellular vRNA. However, in the DCV treated cultures there was an immediate and more rapid drop in the titer that preceded the drop in intracellular vRNA, consistent with the action of an agent that blocked viral assembly or release independent of its effect on intracellular replication.

## Clinical impact of these results

It is important to note that if the HCV half-life is four times shorter than previously thought then the HCV viral production rate must be four times larger than previously thought [7]. Thus the risk of generating mutations conferring drug resistance is also larger than previously thought [8]. Whether the dual mode of action of DCV improves its genetic barrier to resistance is not known but phase 2 clinical trials showed that DCV had to be given in combination with peg/IFN-RBV and or other DAAs to prevent resistance emergence [13].

Blocking assembly/secretion is not a prerequisite for achieving high levels viral suppression. For instance sofosbuvir, a nucleotide analogue polymerase inhibitor [14], was not found to have a significant effect on assembly/secretion (unpublished result) in spite of having a very strong antiviral effectiveness.

Viral kinetic analysis with DCV during longer time periods and with repeated doses will be needed in order to analyze whether a dual mode of action only impacts the early viral kinetics or also leads to more rapid long-term viral decline that may allow for shorter treatment duration. Also, it will be interesting to determine whether other NS5A inhibitors also have dual modes of action, i.e., whether this is a property of the drug class or if this dual mode of action is drug specific and a property of DCV alone.

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