International Hepatology

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

# Mathematical modeling: A tool for selecting agents with complementary modes of action?

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***COMMENTARY ON:***

***Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life. Guedj J, Dahari H, Rong L, Sansone ND, Nettles RE, Cotler SJ, Layden TJ, Uprichard SL, Perelson AS. Proc Natl Acad Sci U S A. 2013 Mar 5;110(10):3991–6. Copyright © 2013. Abstract reprinted with permission from the National Academy of Sciences.***

http://www.ncbi.nlm.nih.gov/pubmed/23431163

***Abstract.*** *The nonstructural 5A (NS5A) protein is a target for drug development against hepatitis C virus (HCV). Interestingly, the NS5A inhibitor daclatasvir (BMS-790052) caused a decrease in serum HCV RNA levels by about two orders of magnitude within 6 h of administration. However, NS5A has no known enzymatic functions, making it difficult to understand daclatasvir's mode of action (MOA) and to estimate its antiviral effectiveness. Modeling viral kinetics during therapy has provided important insights into the MOA and effectiveness of a variety of anti-HCV agents. Here, we show that understanding the effects of daclatasvir in vivo requires a multiscale model that incorporates drug effects on the HCV intracellular lifecycle, and we validated this approach with in vitro HCV infection experiments. The model predicts that daclatasvir efficiently blocks two distinct stages of the viral lifecycle, namely viral RNA synthesis and virion assembly/secretion with mean effectiveness of 99% and 99.8%, respectively, and yields a more precise estimate of the serum HCV half-life, 45 min, i.e., around four times shorter than previous estimates. Intracellular HCV RNA in HCV-infected cells treated with daclatasvir and the HCV polymerase inhibitor NM107 showed a similar pattern of decline. However, daclatasvir treatment led to an immediate and rapid decline of extracellular HCV titers compared to a delayed (6–9 h) and slower decline with NM107, confirming an effect of daclatasvir on both viral replication and assembly/secretion. The multiscale modeling approach, validated with in vitro kinetic experiments, brings a unique conceptual framework for understanding the mechanism of action of a variety of agents in development for the treatment of HCV.*



Chronic hepatitis affects nearly 3% of the world population [1]. The landscape of therapy for hepatitis C virus (HCV) infection, where treatment was still suboptimal until recently, is changing rapidly. Specific proteins involved in the replication of the virus have been identified and targeted by drug development. Among these, we can enumerate non-structural (NS) viral proteins with known enzymatic functions, such as the NS3/4A protease [2,3], and the NS5B polymerase [4,5] but also non-enzymatic targets such as NS5A proteins [6]. The mode of action of HCV drugs targeting these proteins is not always understood. For example, the mode of action of Daclatasvir (BMS-790052), one of the most promising and expected molecules identified as a potent NS5A inhibitor, is not known [6,7]. This is related to the uncertain nature of the molecular mechanisms by which NS5A functions and the absence of direct screening assays for NS5A function.

One means of uncovering an antiviral agent's mode of action is to analyze the kinetics of the response it generates using mathematical modeling. This approach was initiated by Perelson *et al.* in HIV by characterizing the decline in HIV during antiretroviral therapy [8,9] and it was then successfully applied to understand HCV kinetics during therapy [10]. In these models, the infected cell is treated as a "black box" that produces/secretes virus particles, which then either are cleared or infect new target cells, and the effect of treatment is to block virus production from infected cells [10]. Clearly, one limitation of these models is that it does not take into account the stages of the (intracellular) viral lifecycle that are yet the main target of DAAs. In a recent study published in PNAS, Guedj *et al.* introduced a novel generation of models, called "multiscale models" that, in contrast to the standard model, takes into account the dynamics of the intracellular viral RNA and identifies some essential stages of viral replication that can be affected by treatment, namely virion assembly/secretion, viral RNA production, and vRNA degradation (Fig. 1) [11].

This model was applied to the comparison of the viral kinetics observed after initiation of three different classes of agents, namely IFN, telaprevir (a protease inhibitor) and daclatasvir

Keywords: Direct-acting antiviral agents; Mathematical modeling; Viral dynamics.

*Received 24 June 2013; received in revised form 14 July 2013*

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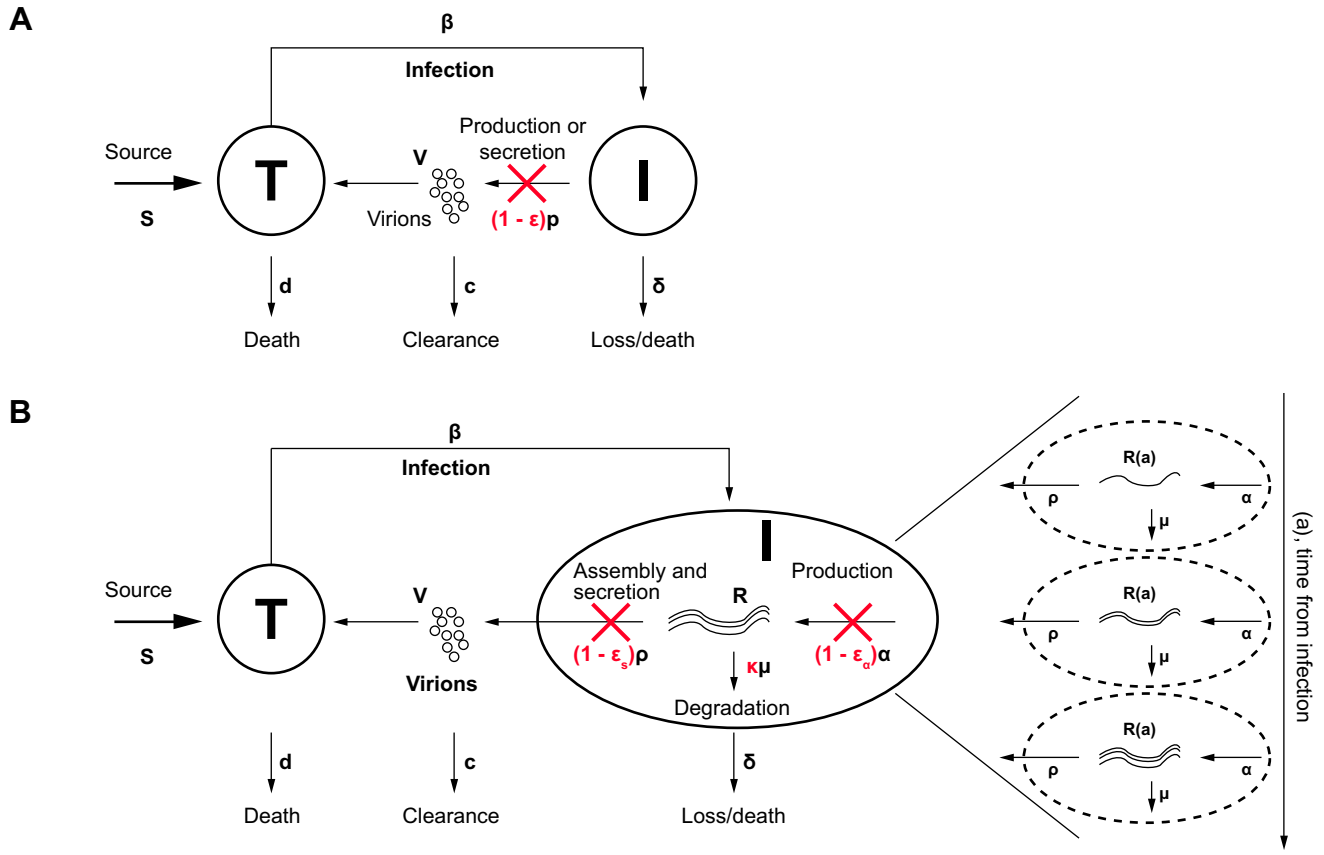


**Fig. 1. Presentation of the standard and multi-scale model of HCV dynamics, and parameter estimates obtained to fit data from patients treated with daclatasvir [11].** In both models, T and I represent target and infected cells, respectively, and V represents virus. Target cells are created and die with constant rates, s and d, respectively, and are infected by virus, V, with constant rate β. Infected cells, I, are lost with constant rate δ, and virus, V, is cleared from serum with constant rate c. (A) The standard model considers only the level of cell infection and virus in the serum. Treatment (parameters in red) acts by reducing the average number of virions produced by infected cells from p to $(1-\varepsilon)p$. Thus, ε represents a global measure of antiviral effectiveness that does not distinguish the stages of intracellular viral replication that are blocked by treatment. The main estimates are $c = 23.3\ d^{-1}$, $\varepsilon = 0.997$, $\delta = 1.06\ d^{-1}$. (B) The multi-scale model was designed to account for essential features of intracellular HCV RNA replication, R, i.e., production, degradation, and assembly/secretion with rates α, μ, and ρ, respectively. The HCV RNA level within an infected cell (dashed circle) is assumed to increase with time since infection and reach a steady state. Treatment (parameters in red) may block HCV RNA production with effectiveness $\varepsilon_\alpha$ and/or virion assembly/secretion with effectiveness $\varepsilon_s$, and/or enhance the degration rate of HCV RNA by a factor κ. The main estimates after fixing $\delta = 0.14\ d^{-1}$, $\mu = 1\ d^{-1}$, $\alpha = 40\ d^{-1}$ are $c = 22.3\ d^{-1}$, $\varepsilon_\alpha = 0.99$, $\varepsilon_s = 0.998$, $\rho = 8.18\ d^{-1}$, $\kappa\mu = 1.46\ d^{-1}$. Figure reprinted from [11], Copyright © 2013, National Academy of Science.

(a NS5A inhibitor) and allowed to puzzle out the mode of action of these drugs. The authors showed that the kinetics observed were dependent on the stages of the viral lifecycle where these drugs acted. While all three agents were found to have a high effectiveness in blocking vRNA production, the reason why HCV RNA declines so rapidly with daclatasvir was due to the fact that daclatasvir, unlike IFN and telaprevir to a lower extent, was extremely effective in blocking viral assembly/secretion, with an effectiveness estimated to 99.8% (*vs.* 39.0% in IFN-treated patients, $p < 10^{-10}$; and 0.94 in telaprevir-treated patients, $p < 10^{-6}$). As a consequence, the number of viruses newly secreted after initiation of daclatasvir is minimal and thus the viral decline observed in serum provides a good model to estimate HCV half-life in serum. Using this approach, Guedj *et al.* could demonstrate that the current estimate of 2.7 h derived from the analysis of viral kinetics during IFN-based treatment were not accurate and now estimate the mean serum half-life of HCV at 45 min. Of note, if HCV half-life is about four times shorter than previously thought, it implies that, in order to maintain a given level of virus, HCV production is also four times larger than initially thought. Thus the risk of generating mutations conferring drug resistance is also larger than previously thought.

The dual mechanism of action of daclatasvir provides a basis to explain why NS5A inhibitors may be good candidates for combination with drugs with complementary mode of actions, such as protease or polymerase inhibitors. Yet, these modeling efforts were based on the analysis of viral kinetics after one single dose of daclatasvir, and important questions on the implications of this dual mode of action remain to be solved. In particular, it is still unclear whether that plays any role in improving the genetic barrier to resistance and how this rapid initial viral decline translates into the long-term viral decline. Clearly, further studies with repeated doses of daclatasvir will be needed to study whether blocking assembly/secretion accelerates only the initial viral decline or also contributes to a faster elimination of the virus in the long run that may allow for shorter treatment duration.

The speed of development of drugs to treat HCV infection is unprecedented and new drugs with high potency, low side effects and requiring short treatment duration are emerging. In this context, mathematical modeling as the one performed by Guedj *et al.*



Please cite this article in press as: Deuffic-Burban S, Yazdanpanah Y. Mathematical modeling: A tool for selecting agents with complementary modes of action?. J Hepatol (2013), http://dx.doi.org/10.1016/j.jhep.2013.07.024

JOURNAL OF **HEPATOLOGY**

is a promising tool to better understand the mechanism of action of new DAAs, such as NS5A and NS5B inhibitors, or a new generation of antiprotease inhibitors [12], rationalizing the combination of these agents and eventually selecting agents with complementary modes of action.

## Conflict of interest

Yazdan Yazdanpanah received travel grants, honoraria for presentations at workshops and consultancy honoraria from Abbott, Bristol-Myers Squibb, Gilead, Merck, Roche, Tibotec and ViiV Healthcare.

Sylvie Deuffic-Burban received grants from Roche, Janssen Pharmaceuticals, and Merck, and consultancy honoraria from Merck, GlaxoSmithKline, and AbbVie.

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Please cite this article in press as: Deuffic-Burban S, Yazdanpanah Y. Mathematical modeling: A tool for selecting agents with complementary modes of action?. J Hepatol (2013), http://dx.doi.org/10.1016/j.jhep.2013.07.024