Supplementary file

Mathematical modeling

Standard model

To predict the time to cure (see term cure boundary in the main text) in our patient under paritaprevir, ombitasvir, dasabuvir, ritonavir (PODr) and ribavirin (RBV or R) therapy, the standard biphasic model, Neumann et al [1], was used:

$$\frac{dI}{dt} = (1-\eta)\beta T_0 V - \delta I$$
$$\frac{dV}{dt} = (1-\varepsilon)\, pI - cV, \qquad \text{(Eq. S1)}$$

where I represents HCV-infected cells and V, free HCV in blood. HCV, V, infects target cells, T_0, with constant rate β, generating infected cells, I, which produce new virions at rate p per infected cell. Infected cells are lost at a rate δ per infected cell and virions are assumed to be cleared at rate c per virion. RBV effectiveness in blocking infection is modeled by a factor (1-η), where η is defined as the drug effectiveness in blocking infection [2, 3]. We assume that RBV does not affect viral production [2, 3], but for PODr a constant effectiveness, ε, was used.

Parameter estimation

We assume the target cell (i.e., hepatocyte) level per ml was constant, i.e., 1×10^7cells/ml (described in [4]), and the initial infected cell level is represented by the steady state pre-treatment level of $I_0=\beta V_0 T_0/\delta$. Due to lack of frequent sampling, especially during the first 2 days after initiation of therapy, the effectiveness of PODr+R in blocking HCV production was set to ε=0.998 as recently estimated [5]. HCV clearance rate was set c=6/day. RBV in blocking HCV entry, η, were fixed to 0.5 as done previously [6]. Parameter β was set to 7×10^{-9} ml/virion/day to allow a low fraction of HCV-infected hepatocytes (~4%), within the range estimated in [7]. The remaining parameter

representing the death/loss rate of infected cells, δ, was estimated by fitting the model with the observed data using Berkeley Madonna (V.8.3).

The time to eradication of the last infected cell is more speculative due to lack of experimental data on the infected cell level. Therefore we focused on the viral cure boundary (i.e., <1 virus copy in the entire extracellular body fluid). Modeling the available HCV RNA measurements using the standard biphasic model suggests a projected time to cure of about 6 weeks after therapy was discontinued, however, the implicit assumption in this case would be that the effect of the antiviral drugs would have lasted during this prolonged period (Fig. S1). Assuming an unlikely, but possible, lower DAA effectiveness, e.g., ε=0.99, predicts higher δ (because the 2nd phase will start from a higher viral load level) that leads to a shorter time to cure of about 3 weeks after therapy was discontinued (not shown).

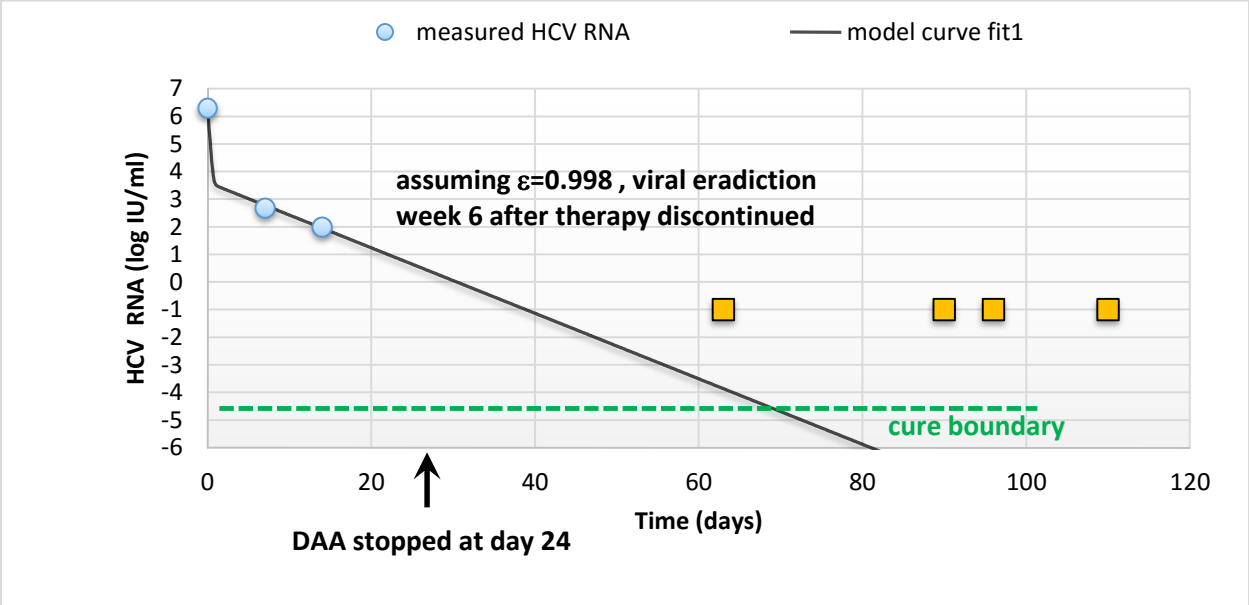

Fig. S1. Standard biphasic model (Eq. S1) fits with measured viral kinetic data (circles) and predicted time to cure. Best curve fit assuming blocking of HCV production of 99.8% (black line) model with observed data. Undetectable HCV RNA, i.e. <12 IU/ml (orange filled squares), measured during therapy, was arbitrarily set to 0.1 log IU/ml

Multiscale model

We next sought to predict the time to cure (see term cure boundary above) in our patient under PODr+R therapy, using the following multiscale model (Eq. 17 in [8])

$$\frac{V(t)}{V_0} = e^{-ct} + (1-\epsilon_s)\frac{c\rho}{N}\left\{\frac{A}{(B-\gamma)\delta(\delta+\gamma-c)}(e^{-ct}-e^{-(\delta+\gamma)t}) + \frac{1}{B+\delta-c}\left(\frac{N}{\rho}-\frac{A}{(B-\gamma)\delta}\right)(e^{-ct}-e^{-(B+\delta)t})\right\}. \quad \text{(Eq. S2)}$$

Where

$$N = \frac{\rho(\alpha+\delta)}{\delta(\rho+\delta+\mu)}, A = (1-\varepsilon_\alpha)\alpha, B = (1-\varepsilon_s)\rho + \kappa\mu .$$

The parameters α, μ and ρ represent the rates of the intracellular HCV RNA (vRNA) production, degradation and assembly/secretion, respectively. Three different antiviral effects of therapy with DAAs are included in the multiscale model (Eq.S2), i.e., blocking vRNA production, α, by a factor $1-\varepsilon_\alpha$, reducing assembly/secretion of virus, ρ, by a factor $1-\varepsilon_s$, and enhancing the rate of vRNA degradation, μ, by a factor κ. Parameters c and δ are as defined in Eq. S1.

Parameter estimation

Because blocking of assembly/secretion and vRNA production by DAAs cannot be estimated without frequent sampling during the first days of therapy, we fixed the following parameters based on [8]: c=22.3/day, ρ=8.18/day, α=40/day, μ=1/day, k=4.94. These values were chosen (Fig. S2) to reproduce a total decline of ~3 logs IU/ml from baseline during the first 2 days of therapy as in [5]. In the multiscale model the final phase slope is $\delta + \gamma$, thus for simplicity γ=0.01/day. The

remaining parameter representing the death/loss rate of infected cells, δ, was estimated by simulating the model with the observed data using Berkeley Madonna (V.8.3).

Analogous to the standard biphasic model, modeling the available HCV RNA measurements with the multiscale model suggests a projected time to cure of about 6 weeks after therapy was discontinued (Fig. S1) with the assumption of lower decline in viral load from baseline during the first 2 days of therapy (~2 log IU/ml) predicting a faster final phase slope that gives rise to a shorter time to cure of about 3 weeks after therapy was discontinued (not shown).

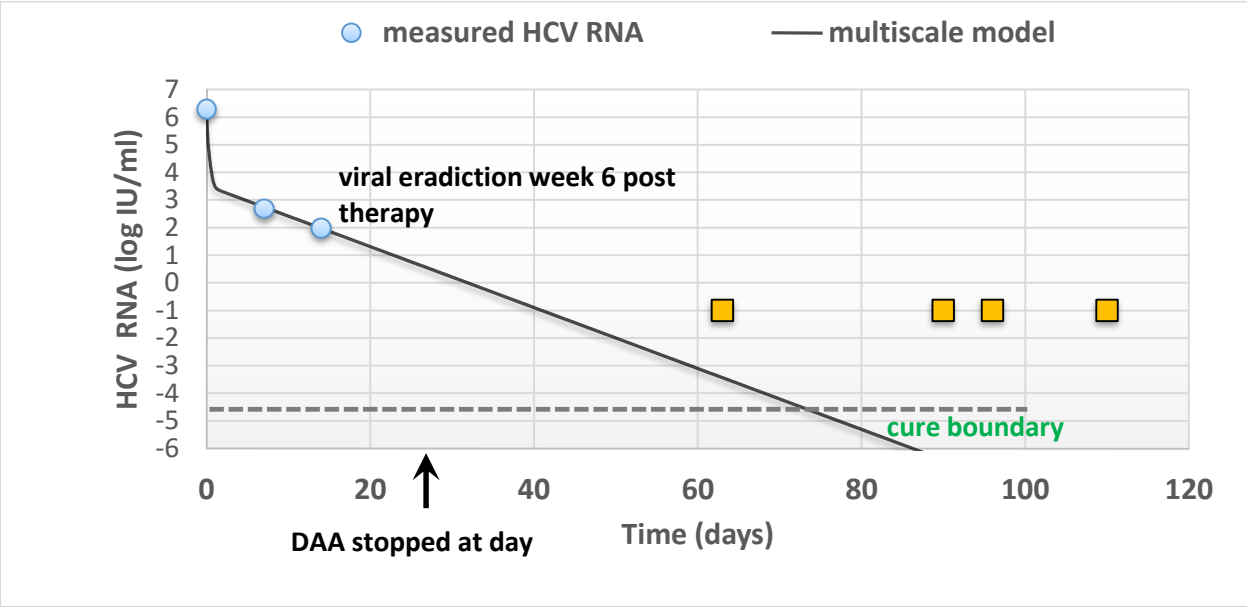

Fig. S2. Multiscale model (Eq. S2) simulation with measured viral kinetic data (circles) and predicted time to cure. Assuming a ~3 log IU/ml drop from baseline during the first 2 days of PODr+RBV, blocking of vRNA production of 99% and assembly /secretion of 90% were assumed. With estimated δ=0.24/day the model (black line) is in agreement with the observed data. Undetectable HCV RNA, i.e. <12 IU/ml (orange filled squares), measured during therapy, was arbitrarily set to 0.1 log IU/ml. Pretreatment total viral load, V_{tot0} was assumed 2,000,000 IU/ml, as measured a week before PODr+R therapy was initiated.

Extended biphasic model that includes infectious and non-infectious virus

To predict cure in our patient before PODr+R was stopped, we developed a model that includes infectious and non-infectious virus:

$$\frac{dI}{dt} = \beta T_0 V_i - \delta I$$
$$\frac{dV_i}{dt} = (1-\varepsilon)\, p_i \exp(-gt) I - cV_i \quad \text{(Eq. S3)}$$
$$\frac{dV_{ni}}{dt} = (1-\varepsilon)\, p_{ni} I - cV_{ni}$$

where I represents HCV-infected cells and V_i *and* V_{ni}, free infectious and non-infectious HCV, respectively, in blood. V_i, infects target cells, T_0, with constant rate β, generating infected cells, I, which produce new infectious and non-infectious virions at rate p_i *and* p_{ni} per infected cell, respectively. Infected cells are lost at a rate δ per infected cell and both V_i and V_{ni} are assumed to be cleared at rate c per virion. DAA-containing regimens such as PODr inhibit HCV replication which effects both V_i and V_{ni} production with a constant effectiveness, ε. However, the ability of DAAs (such as NS5A inhibitors [9]) and RBV [2, 3] to decrease the assembly/secretion of infectious virus results in a gradual increase in the ratio of non-infectious to infectious virus, which is modeled by exp(-gt), where g is a constant and t is time post treatment initiation.

Parameter estimations

We assume the target cell (i.e., hepatocyte) level per ml was constant, i.e., 1×10^7cells/ml (described in [4]), and the initial infected cell level is represented by the steady state pre-treatment level of $I_0=\beta V i_0 T_0/\delta$, where T_0, I_0, and V_{i0}, represent pre-treatment levels of susceptible and infected cells and infectious virus, respectively. Reverse titration studies in chimpanzees have provided direct comparisons between RNA titers and infectious titers of HCV RNA-positive samples [10-12].

Using data from these studies may indicate a pretreatment ratio of V_i and V_{ni} (i.e., p_i/p_{ni}=1/11) in our patient of 1:11 [13]. Due to lack of frequent sampling, especially during the first 2 days after initiation of therapy, the effectiveness of PODr in blocking HCV production was set to ε=0.998 as recently estimated [5]. HCV clearance rate was set at c=6/day [1]. Parameter β was set to 7×10^{-8} ml/virion/day to allow a low fraction of HCV-infected hepatocytes (~4%), within the range estimated in [7]. The remaining parameters representing the death/loss rate of infected cells, δ, and slowing of infectious virion production, g, were estimated by fitting the model with the observed data using Berkeley Madonna (V.8.3).

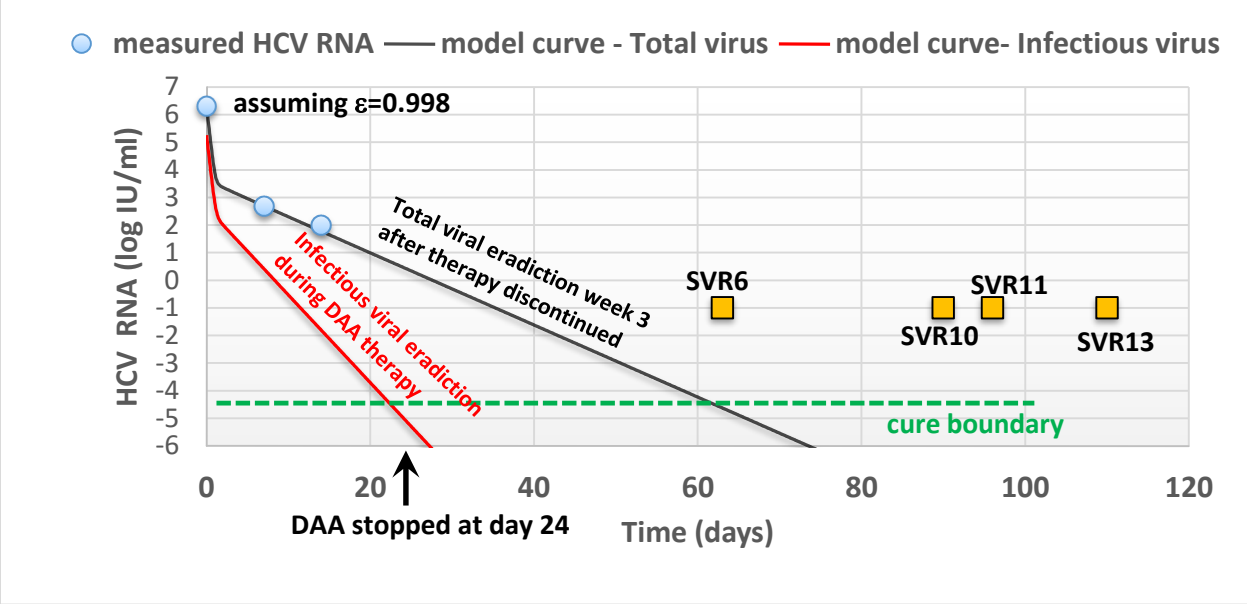

Fig S3. Extended biphasic **Model (Eq. S3) curve fits with measured viral kinetic data and predicted time to cure.** Total viral load (black solid line) was fit to the measured HCV RNA (blue circles) with estimated infected cell loss/death rate of δ=0.3/d. To bring the predicted infectious viral load (red solid line) to cross the cure boundary (horizontal green line) before PODr+R was stopped at day 24 (black arrow), parameter g was estimated as 0.43/d. Undetectable HCV RNA,

i.e. <12 IU/ml (orange filled squares), measured 6, 10, 11 and 13 weeks after DAA discontinued, was arbitrarily set to 0.1 log IU/ml. Pretreatment total viral load, V_{tot0} was assumed 2,000,000 IU/ml, as measured a week before PODr+R therapy was initiated. Pretreatment infectious virus level $V_{tot0}/11$, c=6/d and ε=0.998 were set as explained in Methods.

References

[1] Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ et al. Science 1998;282:103-7.

[2] Dixit NM, Layden-Almer JE, Layden TJ, Perelson AS. Nature 2004;432:922-4.

[3] Dahari H, Ribeiro RM, Perelson AS. Hepatology 2007;46:16-21.

[4] Dahari H, Shudo E, Ribeiro RM, Perelson AS. Mathematical modeling of HCV RNA kinetics. In: Tang H., ed. Hepatitis C Protocols. 2nd ed. NJ: Humana Press, 2009.

[5] Gambato M, Canini L, Lens S, Graw F, Londono MC, Uprichard SL et al. Hepatology 2015;62:1 (Suppl):691A.

[6] Dahari H, Shteingart S, Gafanovich I, Cotler SJ, D'Amato M, Pohl RT et al. Liver Int 2015;35:289-94.

[7] Liang Y, Shilagard T, Xiao SY, Snyder N, Lau D, Cicalese L et al. Gastroenterology 2009;137:1448-58.

[8] Rong L, Guedj J, Dahari H, Coffield DJ, Jr., Levi M, Smith P et al. PLoS Comput Biol 2013;9:e1002959.

[9] Sansone N, Dahari H, Subramanya G, Perelson AS, Uprichard SL. Hepatology 2014;60:4(Suppl):1165A.

[10] Engle RE, Russell RS, Purcell RH, Bukh J. J Med Virol 2008;80:72-9.

[11] Feinstone SM, Alter HJ, Dienes HP, Shimizu Y, Popper H, Blackmore D et al. J Infect Dis 1981;144:588-98.

[12] Major ME, Mihalik K, Puig M, Rehermann B, Nascimbeni M, Rice CM et al. J Virol 2002;76:6586-95.

[13] Cui Q, Gutfraind A, Boodram B, Cotler SJ, Dahari H, Major ME. Hepatology 2015;62:1(Suppl):1114A.