Supplementary Material

Pharmacodynamics of PEG-IFN alpha-2a in HIV/HCV co-infected patients: Implications for treatment outcomes

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Pharmacodynamic model that includes proliferation of uninfected and infected cells

We used a model [1-3] of HCV infection - that predicts various viral decline patterns such as, biphasic, triphasic and flat partial response - to fit patients' data. The equations describing the dynamics of HCV infection and treatment in this model involve uninfected hepatocytes (T), infected hepatocytes (I) and virus (V). Uninfected and infected hepatocytes can proliferate with maximum proliferation rates r_T and r_I, respectively, under the control of a homeostatic process in which proliferation shuts down as the total number of susceptible hepatocytes, irrespective of whether they are infected or not, approaches a maximum number T_{max} [4]. Uninfected hepatocytes are also produced by differentiation from precursors at a constant rate s [5], die at rate d_T per cell and are infected with rate constant β. HCV is produced at rate p per infected cell. PEG-IFN α-2a is assumed to act by partially blocking virion production, with effectiveness ε(t)(see Eq. 4 in the main text). We assume that ribavirin effectiveness $\eta(t)=\eta_{max}(1-\exp(-t/t_a)$, where t_a=5.6 days and η_{max}=0.5, based on the following observations: (i) it takes approximately 4 weeks for ribavirin to reach its steady-state concentration in serum [6], (ii) ribavirin

does not affect the first viral drop in combination with interferon-α [6, 7], and (iii) ribavirin major mode of action is suggested to reducing the rate of infection [2, 8] (see also [9]). Thus, $0 \le \varepsilon(t) \le 1$ and $0 \le \eta(t) \le 0.5$; with 0.5 or 1 meaning 50% or 100% effectiveness, respectively. The corresponding system of differential equations is

$$\begin{aligned} \frac{dT}{dt} &= s + r_T T(1 - \frac{T+I}{T_{\max}}) - d_T T - (1-\eta(t))\beta VT \\ \frac{dI}{dt} &= (1-\eta(t))\beta VT + r_I I(1 - \frac{T+I}{T_{\max}}) - \delta I \\ \frac{dV}{dt} &= (1-\varepsilon(t))pI - cV \ . \end{aligned} \qquad (Eq.S1)$$

The model's steady states at baseline and the baseline fraction of hepatocytes that are HCV-infected with respect to all susceptible hepatocytes , π, are given in the Additional File of [1].

Biphasic-pharmacodynamic model

To fit patients' data in whom a biphasic HCV RNA decline pattern was observed (as explained in Methods in the main text), we used our previously published model [10] of HCV kinetics that allows the estimation of treatment effectiveness, viral clearance and infected cell loss. The solution of our model predicts the evolution of HCV RNA over time, V(t), as:

$$V(t) = V_0(Ae^{-\lambda_1(t-t_0)} + (1-A)e^{-\lambda_2(t-t_0)}) \qquad \text{(Eq. S2)}$$

where

$$\lambda_{1,2} = \frac{1}{2}(c + \delta \pm \sqrt{(c-\delta)^2 + 4(1-\varepsilon(t))(1-\eta(t))c\delta}) \quad \text{and} \quad A = \frac{\varepsilon(t)c - \lambda_2}{\lambda_1 - \lambda_2} \ .$$

Equation (S2) predicts that the viral load under treatment will always decrease to the uninfected steady state following an initial rapid viral decline (first phase) followed by a slower decay (second phase), with rates λ_1 and λ_2, respectively. The parameter t_0 is a delay, corresponding to the time it takes between the initiation of drug treatment and its effect in reducing HCV RNA. The solution is valid for $t > t_0$. For $t < t_0$, the solution is $V(t)=V_0$, where V_0 is the baseline HCV RNA. $\varepsilon(t)$ and $\eta(t)$, c and δ are as explained above for Eq. S1.

Fitting equation S1 to the experimental data

The parameters t_0, V_0, and c (for each patient) were estimated by equation 4 given in Neumann et al.[10] as explained in the main text. In addition, as previously described[1], we fixed r_T=3 day^{-1}, T_{max}=7.5x10^6 cell/ml, d_T=3.5x10^{-3} day^{-1} and s=1 cell/day/ml. The remaining parameters δ, r_I, p, Ec_{50}, and β were estimated for each patient (Table S1), using Berkeley Madonna (http://www.berkeleymadonna.com). As previously described [1], we let δ, r_I, p, and β to be estimated within the following parameter space: $0.2 \le r_I \le 3.0$ day^{-1}, $1 \le p \le 15$ day^{-1}, $d_T \le \delta \le 3$ day^{-1}; $10^{-8} \le \beta \le 10^{-6}$ ml/day/virions. Fitting results for the first 5 weeks of treatment are shown in Fig. S1.

Baseline characteristics and viral kinetics of the *discontinued* patients

Five patients have discontinued therapy after 11 or 12 weeks (three asked to leave, one went to jail, and one died for a cause not related to the study). Their baseline characteristics and viral kinetics are shown in Table S2 and Fig. S2, respectively.

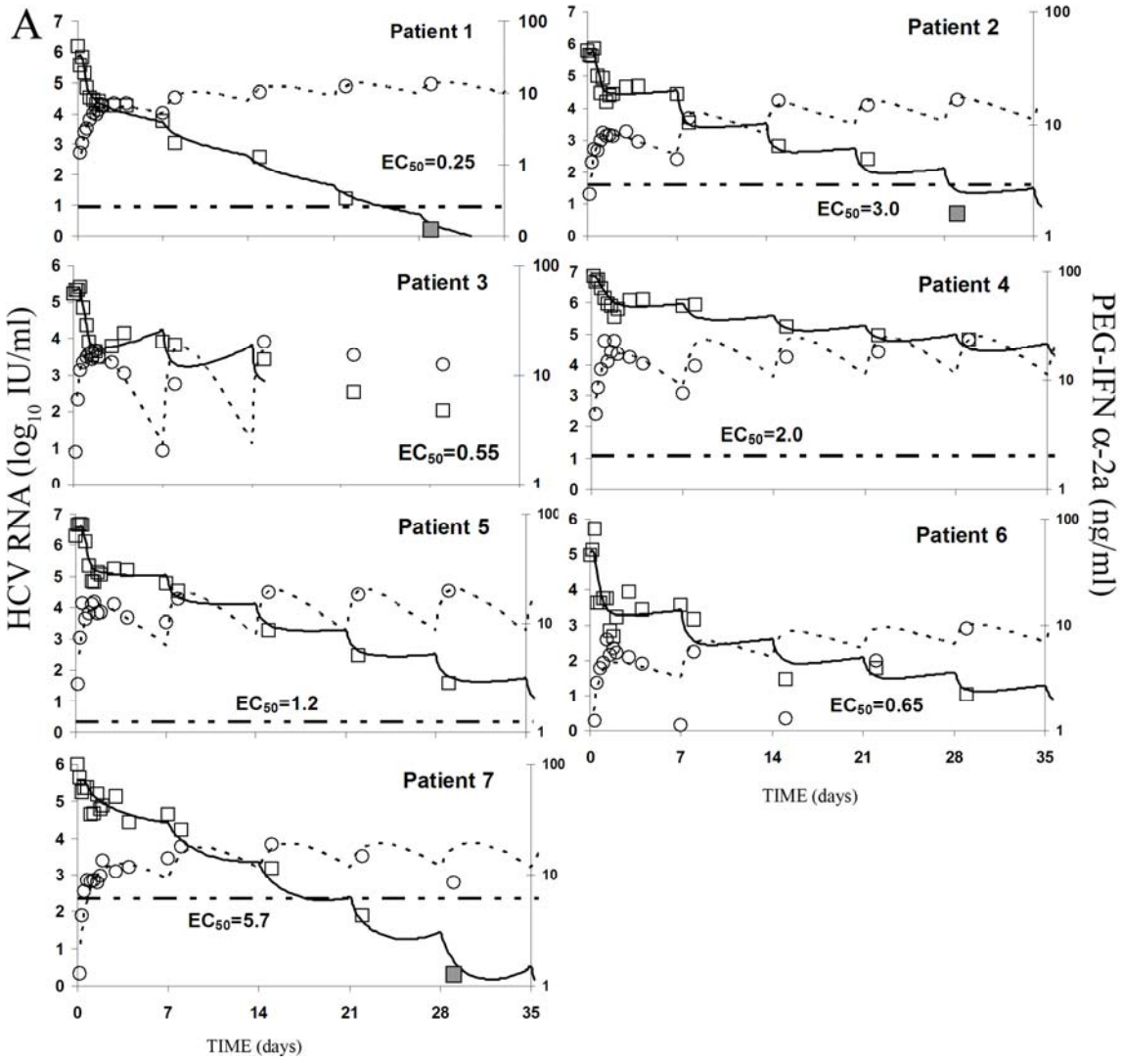
Table S1. Parameter estimates obtained by fitting equation S1 to patients' data.

Patient No.	r_I	β	δ	p	n	π	Ec_{50}	ε_{7max}	ε_{max}
1*	0.96	9.9	0.34	0.90	1.0	86%	0.19	0.974	0.985
2*	3.00	1.7	0.18	0.80	2.6	39%	2.60	0.953	0.993
3*@									
6*	2.97	1.8	0.14	0.60	2.0	13%	0.67	0.987	0.996
7*	2.97	1.9	0.46	1.97	1.0	13%	6.90	0.632	0.676
4*	0.51	2.6	0.27	4.10	1.0	99%	1.97	0.908	0.925
5*	0.57	3.4	0.30	7.40	1.2	95%	0.66	0.975	0.983
Median (IQR)	**2.0 (2.4)**	**2.3 (3.3)**	**0.29 (0.20)**	**1.4 (4.8)**	**1.1 (1.2)**	**63 (83)**	**1.3 (3.1)**	**0.964 (0.140)**	**0.984 (0.130)**
8	2.97	3.9	0.18	0.38	1.0	86%	1.08	0.856	0.911
9	2.97	0.3	0.26	6.20	1.0	16%	1.70	0.868	0.884
13	1.38	9.9	0.26	1.00	1.0	99%	1.40	0.917	0.935
10	0.51	0.2	0.07	2.95	1.0	6%	5.71	0.672	0.759
11	0.00	1.6	0.11	0.70	2.0	45%	2.90	0.938	0.972
12	0.63	1.0	0.10	1.20	1.0	13%	21.46	0.380	0.480
14	1.26	0.9&	0.11	2.60&	1.0	99%	13.40	0.469	0.624
15@									
16	2.22	0.7	0.11	0.89	1.0	98%	0.78	0.946	0.961
17	2.67	0.5	0.11	3.10	1.0	98%	2.27	0.856	0.892
18**	NF	NF	NF	NF	NF	NF	NF	NF	NF
19**	NF	NF	NF	NF	NF	NF	NF	NF	NF
20	0.75	1.1	0.15	5.20	1.0	99%	3.60	0.757	0.841
21**	NF	NF	NF	NF	NF	NF	NF	NF	NF
Median (IQR)	**1.3 (2.1)**	**1.0 (1.7)**	**0.11 (0.09)**	**1.9 (2.8)**	**1.0 (0.2)**	**92 (84)**	**2.6 (6.3)**	**0.856 (0.300)**	**0.888 (0.220)**
P value	**NS**	**0.034**	**0.01**	**NS**	**NS**	**NS**	**NS**	**0.039**	**0.039**

β, viral infection rate [10^{-7}mL/virions/day]; r_I, HCV-infected cell proliferation rate constant [1/day]; p, viral production rate constant [virions/cell/day]; EC_{50}, PEG-IFN concentration that decreases HCV production by 50%; n, Hill coefficient; ε_{7max}, maximum effectiveness during first dose of PEG-IFN; ε_{max}, 4 to 12-week PEG-IFN effectiveness. π, estimated baseline percentage of hepatocytes that are HCV-infected; $\pi = \bar{I}/(\bar{T}+\bar{I})$, where an over bar denotes a baseline value (see Additional File of our recent publication[1]). V_0, c, and t_0 values for each patient are shown in Table 3 in the main text.

&, these parameter values were fixed during fit to allow model convergence. NF, model cannot not be fitted to the data. NA, in these patients PEG-IFN concentration declined from day 15 (see Fig. 2 in main text). @, in these patients PEG-IFN concentration dropped from day 15, thus parameter estimates were not performed with Eq. S1 but with Eq. S2 (see Table 3 in the main text).

Figure S1. PEG-IFN-α-2a serum concentrations and HCV-RNA levels during the first five weeks of treatment in (**A**) SVRs and (**B**) non-SVRs. Graphs show drug concentration data (circles) and best-fit theoretical curve (Eq. 3 (see main text), dashed line; right axis, ng/ml) and HCV RNA data (squares) and best-fit curve (solid line) (left axis, $\log_{10}$ IU/mL) from our combined triphasic or biphasic pharmacodynamic models (Eqs. S1 or S2, respectively; see also Table 3 in the main text). Estimated EC_{50} is represented by horizontal dotted-dashed line.



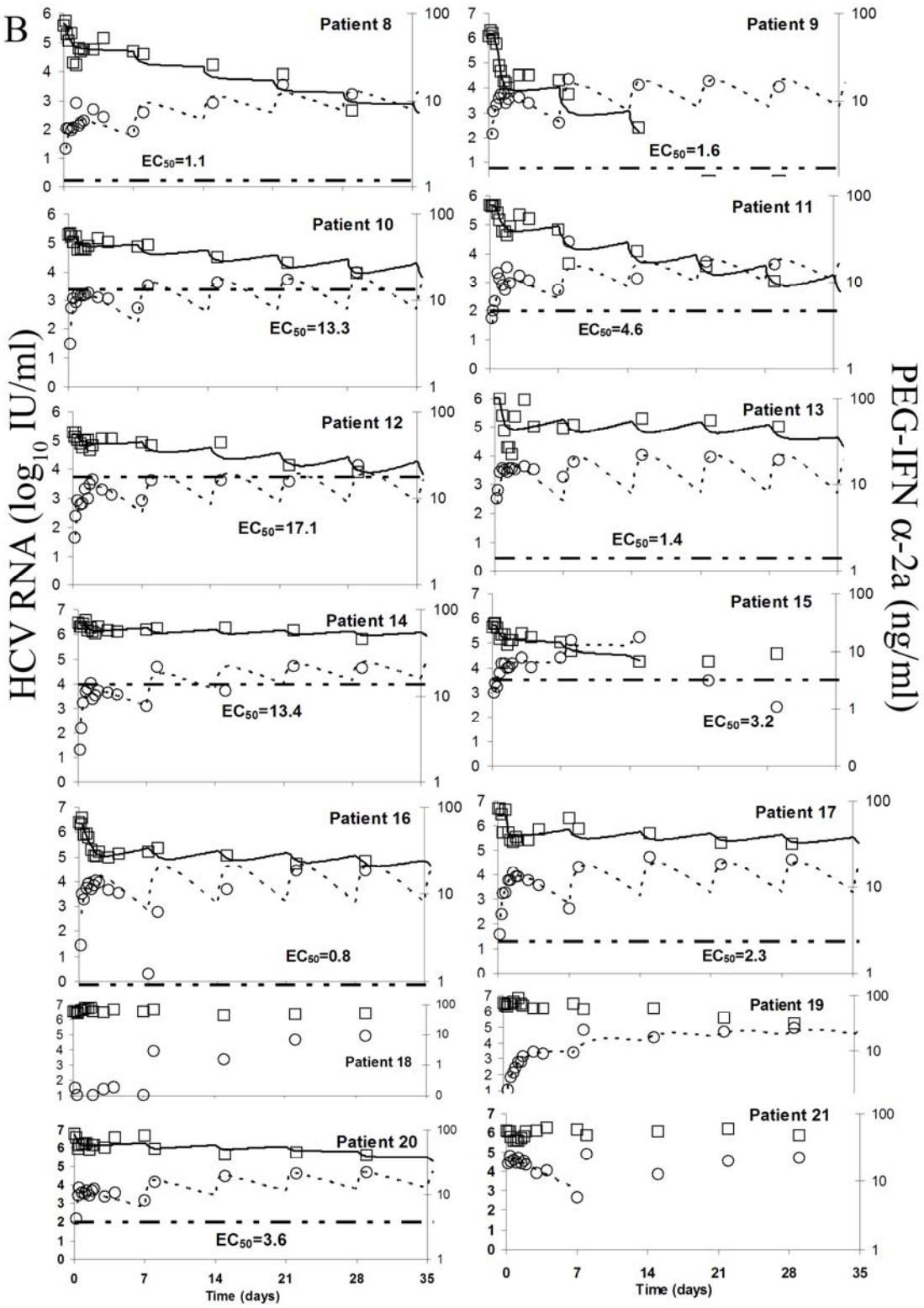
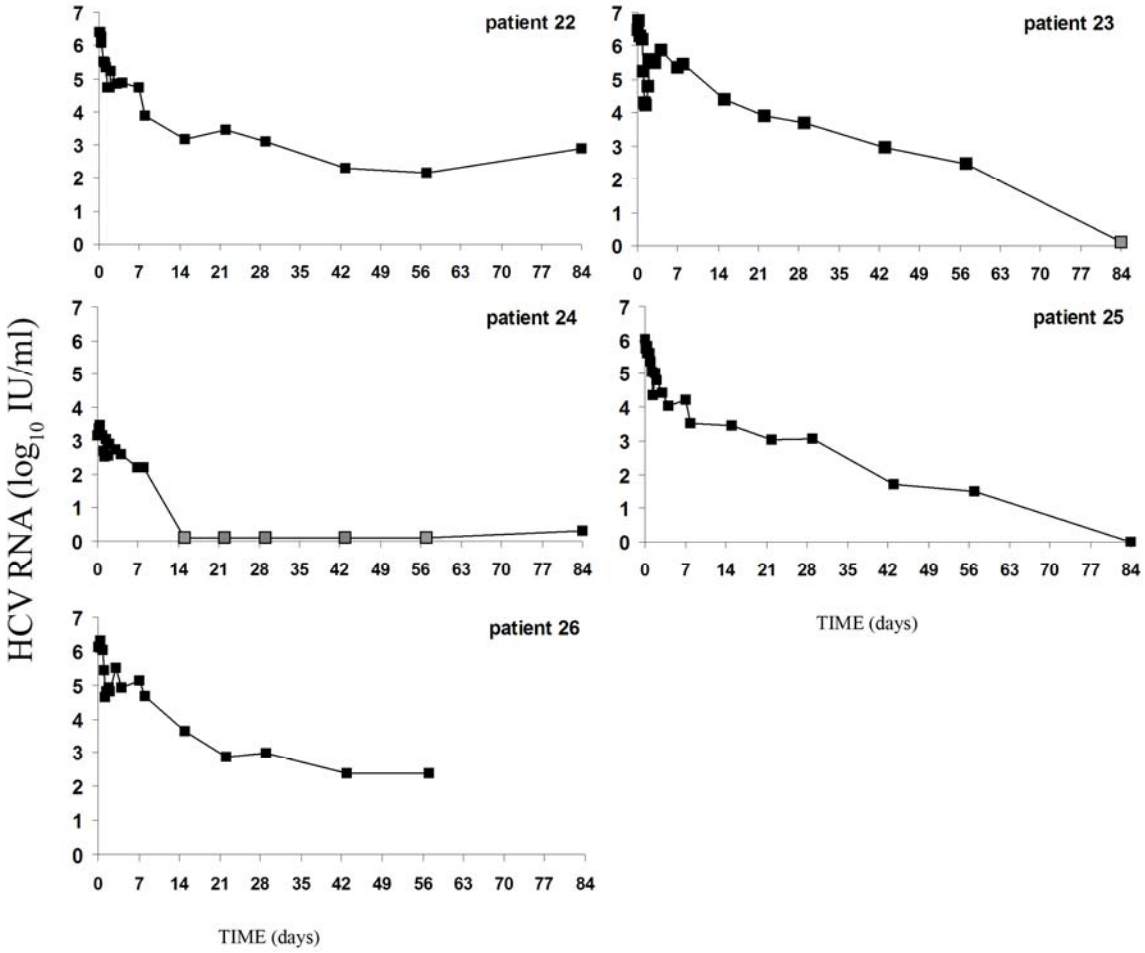

B
Patient 8
EC50=1.1
Patient 9
EC50=1.6
Patient 10
EC50=13.3
Patient 11
EC50=4.6
Patient 12
EC50=17.1
Patient 13
EC50=1.4
Patient 14
EC50=13.4
Patient 15
EC50=3.2
Patient 16
EC50=0.8
Patient 17
EC50=2.3
Patient 18
Patient 19
Patient 20
EC50=3.6
Patient 21
HCV RNA (log10 IU/ml)
PEG-IFN α-2a (ng/ml)
Time (days)
Time (days)

Table S2. Baseline Characteristics of the *discontinued* patients.

Patient No.	Genotype	race	Age (yr)	gender	Weight (kg)	CD4 (cells/mm^3)	Metavir
22	1	W	38	M	71	486	F1A1
23	1	B	47	M	78	524	F1A1
24	3	B	39	M	67	455	F4A3
25	3	W	49	M	71	424	F2A3
26	3	B	34	M	65	658	F1A1

W, white; B, black; M, male.

Figure S2. HCV RNA levels during 12 weeks of treatment. Gray squares indicate undetectable HCV RNA (<10 IU/ml). Solid lines were used to emphasize phases of viral decline.



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