HCV kinetic and modeling analyses indicate similar time to cure among sofosbuvir combination regimens with daclatasvir, simeprevir or ledipasvir

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Supplementary materials and methods

(1) Estimation of the pre-treatment frequency of infected cells

To estimate the pre-treatment frequency of HCV infected hepatocytes in each patient, we used the following previously determined relationship with measured serum viral load of a patient (1). Let *N* denote the total number of hepatocytes in the liver, *f* the pre-treatment frequency of infected cells, hence, *I=fN*, denotes the number of infected hepatocytes in the liver. We assume that each infected hepatocyte has an average intracellular HCV RNA content of *H* and that viral RNA is packaged and exported from infected cells at a rate *ρ*. Denoting the *physiological* clearance rate of extracellular virus by c_p, the serum viral load of a patient, *V*, can be estimated by

$$V = \frac{\rho f N H}{c_p \gamma} \qquad (Eq.S1)$$

Hereby, γ denotes a scaling factor to account for the fact that *V* is measured per ml of serum, while $\rho fNH/c_p$ defines the total number of virions in the human body. As the total extracellular fluid volume for a 70kg individual is estimated to be ~15L (2), we define *γ* dependent on the weight, *w*, of the patient as

$$\gamma(w) = 0.95 \frac{15l}{70kg} w \qquad (Eq.S2)$$

The correction factor of 0.95 was chosen as we observed previously that estimates of the viral load based on the measured frequency of infected cells in liver biopsy samples and their amount of

intracellular HCV RNA was usually 0.3 logs (or 5%) of the actually measured viral load (1). With Eq.S1 and Eq.S2, the pre-treatment frequency of infected cells can be formulated as

$$f(w,V) = \frac{c_p \gamma(w)}{\rho N H} V \qquad (Eq.S3)$$

To estimate the pre-treatment frequency of infected cells per patient based on their viral load and the body weight, we performed 10,000 replicates of Eq.S3, assuming the following parameterization for ρ, c_p, N, and H. The viral export rate and the physiological viral clearance rate are estimated to be ρ=8.18 day^{-1} [4.65, 11.71] (3), and c_p=22.3 day^{-1} [18.97, 25.63] (3), respectively, and we varied both of them uniformly within their confidence intervals. In addition, the total number of hepatocytes, N, is assumed to be between ~10^{11} – 2x10^{11} cells (4, 5). Analyzing liver biopsy samples, Wieland et al. (6) observed a positive correlation between the average amount of intracellular viral RNA, H, and the serum viral load of a patient. Based on these data, we approximated that H increases exponentially with increasing log10 serum viral load according to $H(V) = exp(\alpha[V-1])$ with $\alpha \epsilon [0.08, 0.27]$ (Supplementary Fig. A).

Using these parameterizations, the pre-treatment frequency of infected hepatocytes for an individual of ~70kg based on the serum viral load is shown in Supplementary Table A and Supplementary Fig AB. Due to the uncertainty in the upper bound of these estimates (especially in viral load > 6 log IU/ml), i.e. the assumed exponential relationship for H(V) might be underestimating the true intracellular viral RNA content for these viral loads (6), only the minimum estimates were used. Individual estimates for each patient are shown in Table 2.

(2) Description of the nonlinear mixed effect models.

Nonlinear mixed effect models (or population approach) were first developed to study the pharmacokinetics (PK) of drugs (7). This method allows a description of population characteristics (mean parameters) as well as the inter-individual variability (IIV) (8). In this method, a function *f* describing the variables being modeled, e.g., the viral load or drug concentration, depends nonlinearly on θ_i, a vector of the *p* parameters of subject *i*. A vector ξ_i representing the times at which samples are collected from subject *i*, $\xi_i = (t_{i1}; t_{i2};\ldots; t_{in})$, is also considered. The statistical model for subject *i* is then given by:

$$y_i = f(\theta_i;\xi_i) + ae_i$$

where y_i is a vector with n_i observations of subject *i*, with *i* varying from 1 to *N*, ε_i is the vector of the residual errors which is the part of the observations unexplained by the model *f*. It is assumed that the errors ε_i are independent from one observation to another and that their distribution is Gaussian $\varepsilon_i \sim N(0; I_{ni})$, where I_{ni} is an identity matrix of dimension n_i. *a* is a parameter characterizing the error model variance.

In nonlinear mixed effect models, the model *f* is common to all the subjects, but the vector of parameters θ_i for subject *i* may vary from one subject to another. The inter-individual variability is modeled with the vector of random effect parameters θ_i. The vector of parameters θ_i for the subject *i* can then be expressed as a second-level model which links with the function g, the vector of fixed effect parameters β common for all subject and the vector of random effects η_i specific for subject i: $\theta_i = g(\beta;\eta_i)$. The vector of random effect is assumed to follow a Gaussian distribution $\eta_i \sim N(0; \Omega)$, η_i and ε_i are assumed to be independent for subject *i* and $\eta_i | \varepsilon_i$ is assumed independent from one subject to another. Ω is the matrix of random effect variance. Here, the function *g* is an exponential model. The vector of parameters is hence written as $\theta_i = \beta e^{\eta_i}$.

The residual error measures the difference between predictions and observations. The model used was: $y = f + a\varepsilon$. *y* is the observed response, *f* is the model function, *a* is the additive error term, and the error ε is normally distributed following $N(0,\sigma^2)$ where σ^2 is the variance of the residual variability.

(3) Parameter estimation method.

HCV RNA data including the first viral load below the limit of quantification (<15 IU/ml) or below the limit of detection was used for model fits, using a population approach, whereas the subsequent observations were truncated. In order to accurately estimate the model parameters we excluded two subjects (R1 and H10) with less than 2 samples above the limit of quantification. The viral clearance rate constant was fixed to c=6/day (Table 2). The population parameters: baseline viral load (V_0), ε and δ and their inter-individual variability (IIV) estimates were obtained using a maximum-likelihood method implemented in MONOLIX version 4.3.2 (Lixoft, Orsay, France), which uses the stochastic approximation expectation-approximation (SAEM) algorithm (9) to estimate population parameters. The model was fit to log10 viral load. HCV genotype and patient type (treatment naïve vs. non-responder) were included as covariates in the model to study their effect on the parameters. Individual parameters were estimated using the empirical Bayes method (10). The BIC (11) was used to compare various models.

(4) Assuming cure as <1 virus copy in the entire extracellular fluid and <1 infected hepatocyte.

Further analysis predicted that the total mean time to reach a definition of cure defined as not only <1 virus copy in the entire extracellular fluid, but also <1 infected hepatocyte was 8.6 weeks [95%CI: 7.7 to 9.6 weeks]. Using time to this dual endpoint as the threshold to achieve cure predicts that, 20 (37%) subjects reached cure after 6 weeks of therapy, 4 (7%) subject after 8 weeks, 19 (35%) subjects after 10 weeks, 3 (6%) subject after 12 weeks, and 8 (15%) subjects with more than 12 weeks of therapy (Fig. SB). Restricting the analysis to patients with HCV genotype-1 (n=50) did not change the pattern of results. However, since all subjects achieved SVR but one relapser (L1, Table 2 and Fig. 2), the prediction of more than 12 weeks of treatment to achieve <1 infected cell in 7 individuals (S3, D5, D11, D14, L5, L13 and L18 - Table 2) who achieved SVR with 12-week therapy is an overestimate.

Supplementary Table A: Estimated frequency of infected cells based on Eq.S3 and the viral load for an assumed body weight of 70kg. Results are based on 10^4 bootstrap replicates using the parameterization as described in the text.

Viral load (Log10)	Median (in %)	Min-Max (in %)	10-90% (in %)	25-75% (in %)
4	0.2	[0.1, 0.6]	[0.1, 0.3]	[0.1, 0.2]
4.5	0.4	[0.1, 1.6]	[0.2, 0.8]	[0.3,0.6]
5	1.3	[0.4, 5.2]	[0.8, 2.2]	[1.0, 1.7]
5.5	3.6	[1.1, 14.5]	[2.1, 6.5]	[2.7, 4.9]
6	10.4	[3.3, 43.9]	[6.0, 19.1]	[7.6, 14.3]
6.5	29.5	[9.1, 100]	[16.7, 54.9]	[21.6, 40.9]
7	84.2	[24.8, 100]	[46.5, 100]	[60.8, 100]
7.5	100	[71.8, 100]	[100, 100]	[100, 100]

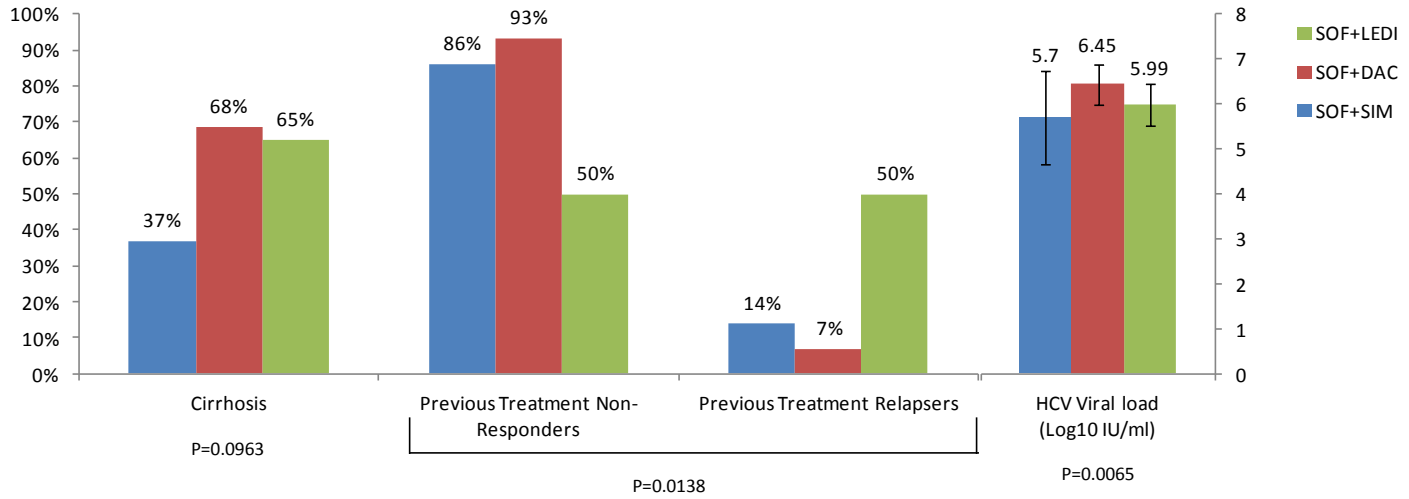
Supplementary Table 1. Population parameter estimates

Parameter type	***ε***	***V_0*** **(log_{10} IU/mL)**	***δ*** **(d^{-1})**
Estimates (s.e.)	0.997 (0.0008)	6.07 (0.15)	0.406 (0.033)
IIV % (s.e. %)	-	8 (1)	46 (6)

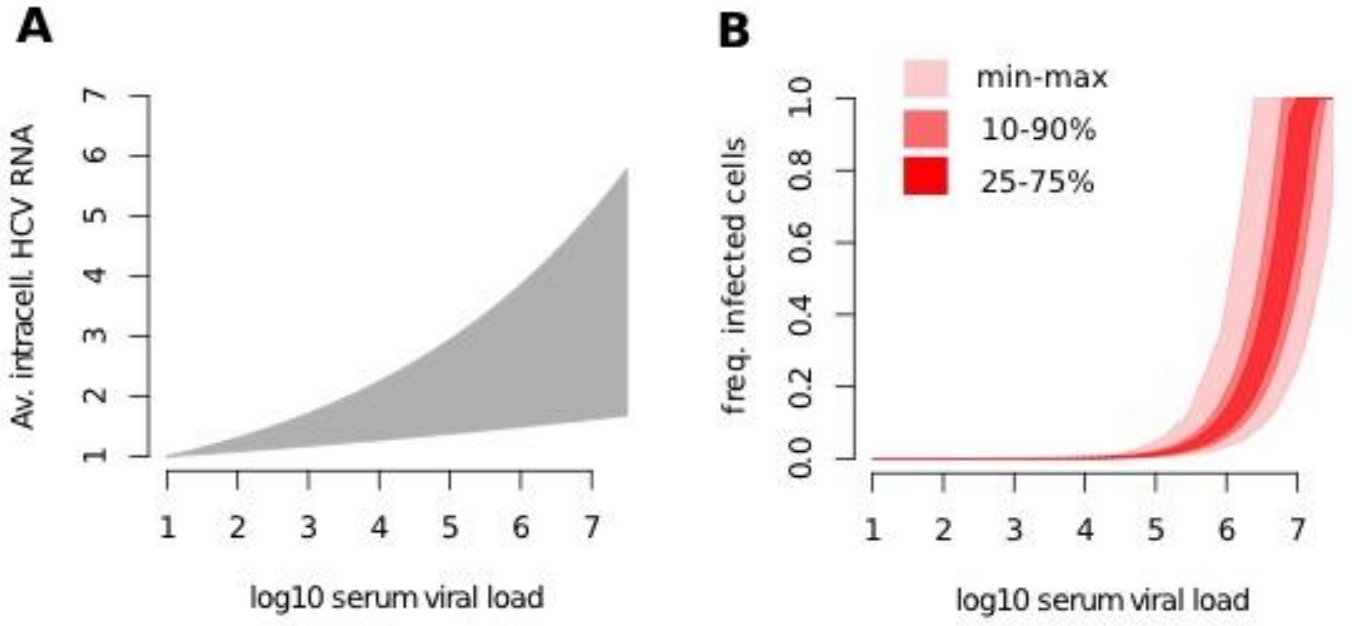
ε treatment effectiveness in blocking viral production; V_0, baseline HCV RNA; ***δ*** infected-cell loss rate; s.e.: standard error; IIV: Inter-individual variability. HCV clearance from circulation, c, was fixed to 6.0/day consistent with (12, 13).

Supplementary Table 2.

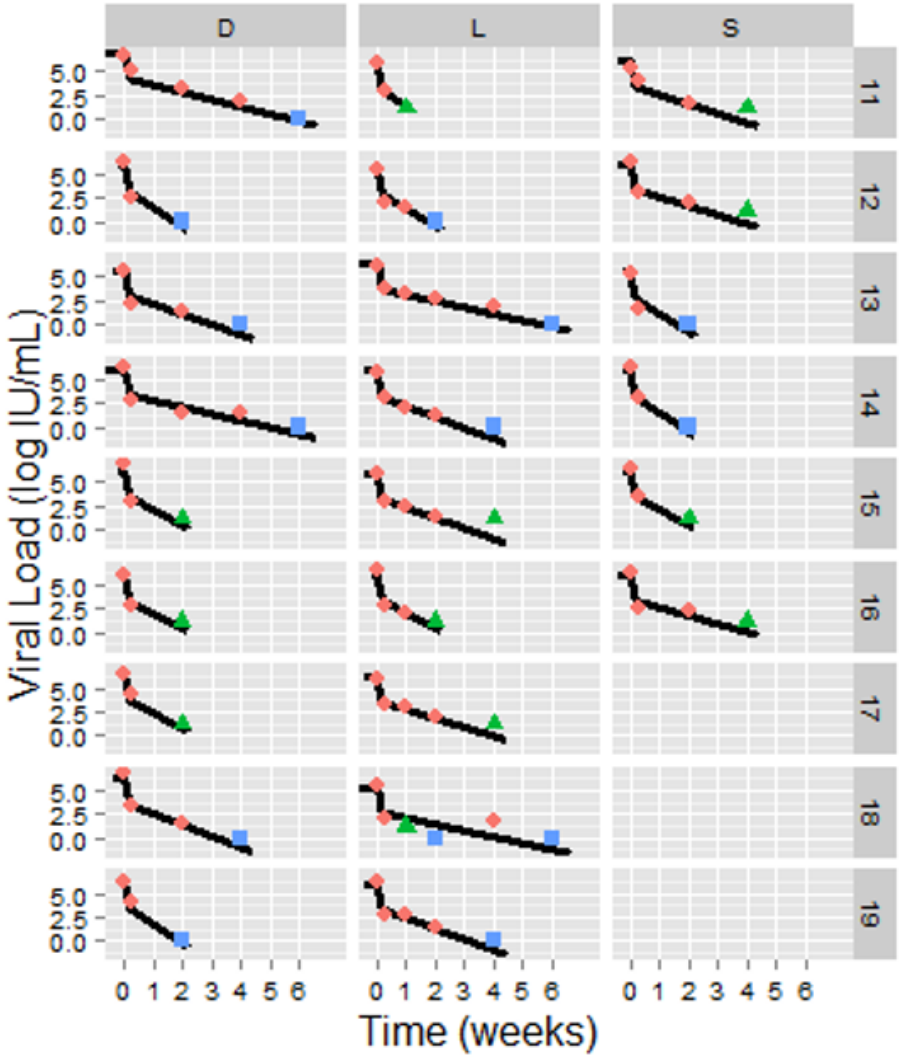
Patients Characteristics	Cohort	NR	Relapsers
Previous Antiviral Treatment – N(%)	N=58	(n=33)	(n=11)
Naïve	14 (24%)	-	-
PegIFN-RBV	29 (50%)	26 (79%)	3 (27%)
PegIFN-RBV + Telaprevir	8 (14%)	1 (3%)	7 (64%)
PegIFN-RBV + Boceprevir	1 (<2%)	1 (3%)	
Protease Inhibitor	4 (7%)	4 (12%)	0
PegIFN alone	1 (<2%)	0	1 (9%)
Sofosbuvir + RBV	1 (<2%)	1 (3%)	0



Supplementary Fig. 1. Baseline characteristics distribution between treatment groups. Vertical black lines represent one standard deviation.

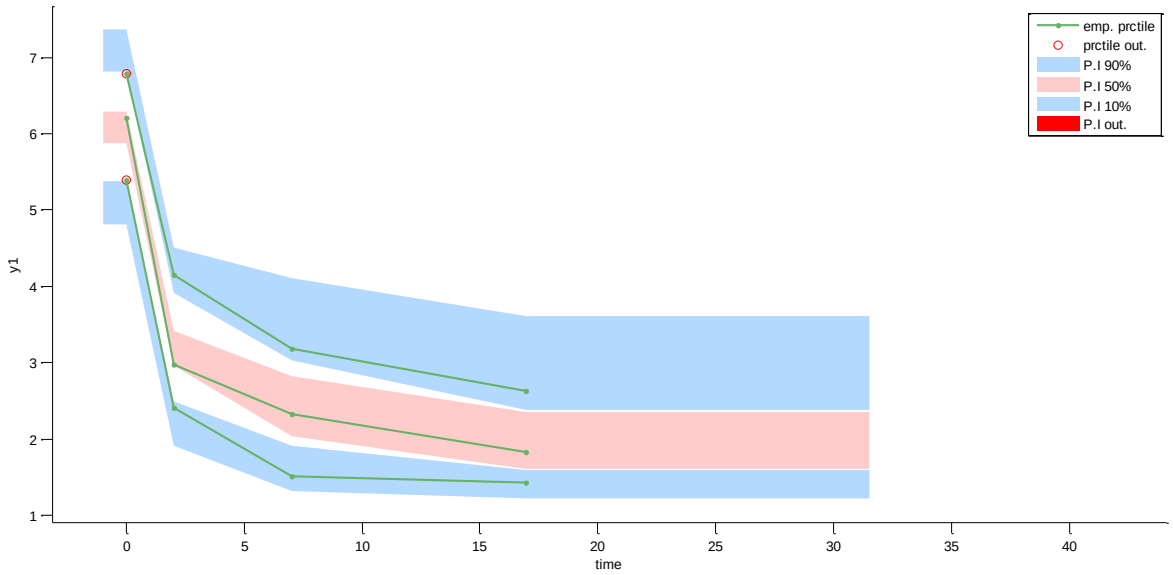


Supplementary Fig. A. (A) Approximated relationship between the serum viral load and the average intracellular HCV RNA content based on the data of Wieland et al., (6) defined by $H(V) = exp(\alpha[V-1])$ with $\alpha \epsilon [0.08, 0.27]$. (B) Range of estimated frequencies of infected cells dependent on the serum viral load according to Eq.S3 based on 10^4 replicates.



Supplementary Fig. 2. . HCV kinetics observed in the remaining 24 subjects (30 more subjects are shown in main text - Fig. 2) treated with sofosbuvir in combination with daclatasvir (D), ledipasvir (L) or simeprevir (S). Quantifiable HCV (Pink circles); HCV <15 IU/ml (Green triangles) and target not detected (blue squares). Biphasic model (Eq. 1) fitting curves are shown with solid lines.

A



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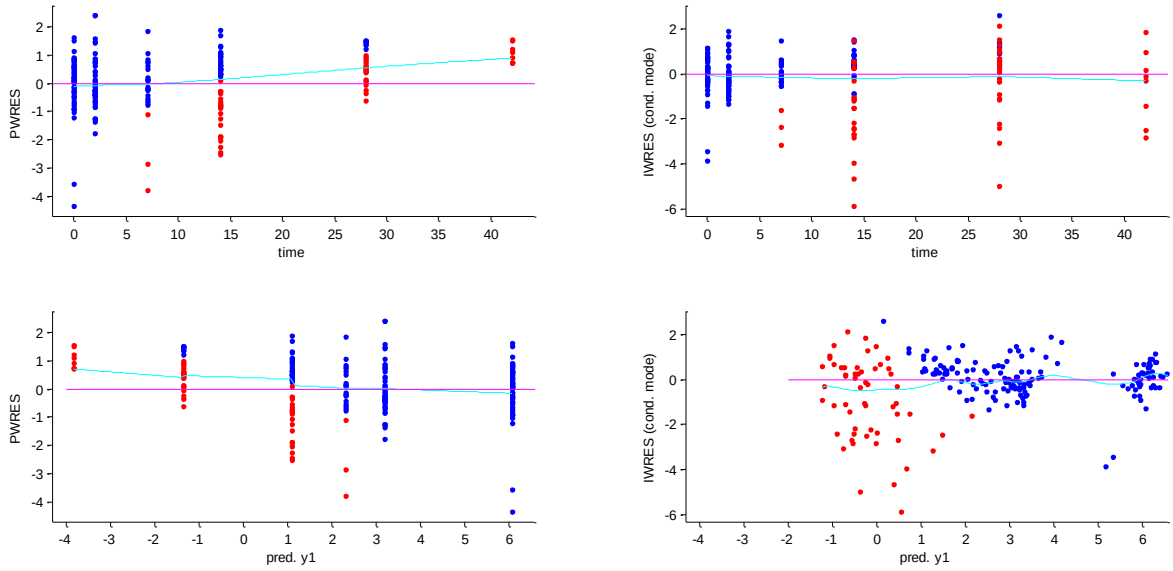
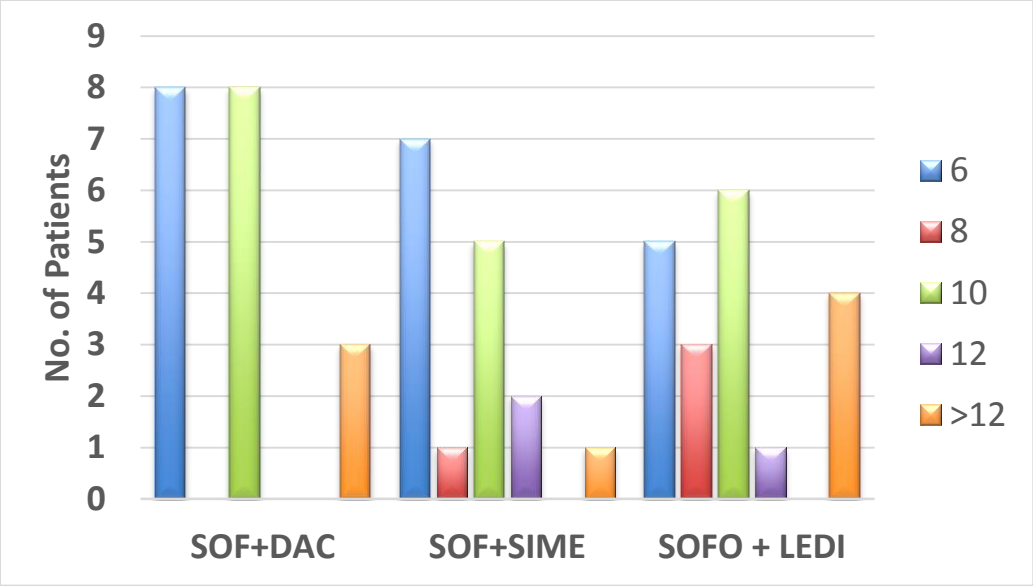


Supplementary Fig. 3. Goodness of fit plots. A) Visual predictive check represents the empirical percentiles (10%, 50% and 90%, green lines) and the 90% confidence intervals for these percentiles computed from 500 simulations of the observations based on the model, according to the original study design. B) Residuals plots. Observations are shown by dots and BLQ data are in red. The

upper panels show population weighted residuals (PWRES) (left panel) and individual weighted residuals (IWRES) (left panels) depending on time. The lower panels PWRES (left panel) and IWRES (right panels) depending on predictions.



Supplementary Fig. 4. Projected treatment duration (weeks) to reach cure based on viral and infected cell cure boundary defined as <1 virus copy and <1 infected cell in patient. In 8 patients the projected time for both virus and infected cell elimination form the body exceeded 12 weeks. SOF, sofosbuvir; DAC, daclatasvir; SIME, simeprevir; LEDI, ledipasvir.

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