10th December 2015

Exploring Viral Kinetics in Hepatitis

Valeria Cento

EUROPEAN HIV HEPATITIS CO-INFECTION (EHHC)

CONFERENCE

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COMPETING INTEREST OF FINANCIAL VALUE ≥ £1,000:	
Speaker Name	**Statement**
Valeria Cento	None declared
Date	December 7, 2015

Hepatitis C Viral Dynamics in Vivo and the Antiviral Efficacy of Interferon-α Therapy

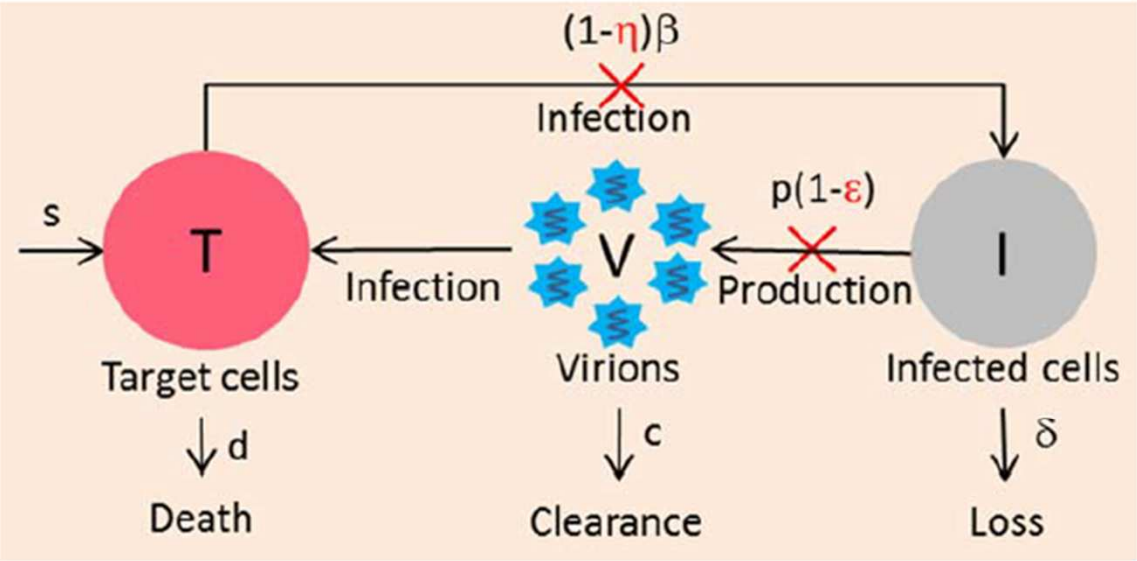
Avidan U. Neumann,*† Nancy P. Lam,*‡ Harel Dahari, David R. Gretch, Thelma E. Wiley, Thomas J. Layden, Alan S. Perelson

To better understand the dynamics of hepatitis C virus and the antiviral effect of interferon-α-2b (IFN), viral decline in 23 patients during therapy was analyzed with a mathematical model. The analysis indicates that the major initial effect of IFN is to block virion production or release, with blocking efficacies of 81, 95, and 96% for daily doses of 5, 10, and 15 million international units, respectively. The estimated virion half-life ($t_{1/2}$) was, on average, 2.7 hours, with pretreatment production and clearance of 10^{12} virions per day. The estimated infected cell death rate exhibited large interpatient variation (corresponding $t_{1/2}$ = 1.7 to 70 days), was inversely correlated with baseline viral load, and was positively correlated with alanine aminotransferase levels. Fast death rates were predictive of virus being undetectable by polymerase chain reaction at 3 months. These findings show that infection with hepatitis C virus is highly dynamic and that early monitoring of viral load can help guide therapy.

Neumann AU et al., Science 1998

Standard model of HCV infection

The standard model considers only the **level of cell infection and virus in serum**. Treatment acts by reducing the average number of virions produced by infected cells from p to $p(1 - \varepsilon)$. Thus, ε represents a global measure of antiviral effectiveness that does not distinguish the stages of intracellular viral replication that are blocked by treatment.



Nguyen THT and Guedj J, CTP Pharmacometrics Syst. Pharmacol. 2015

Standard viral kinetic model. Target cells (T) are produced at rate s, die with death rate d, and become infected cells (I) with infection rate b by free virus (V). Infected hepatocytes die with rate constant d. V are released from infected cells at a rate of p and are cleared with a rate c. Treatment is assumed to act by blocking new infection with an effectiveness g, or by blocking virion production with an effectiveness e.

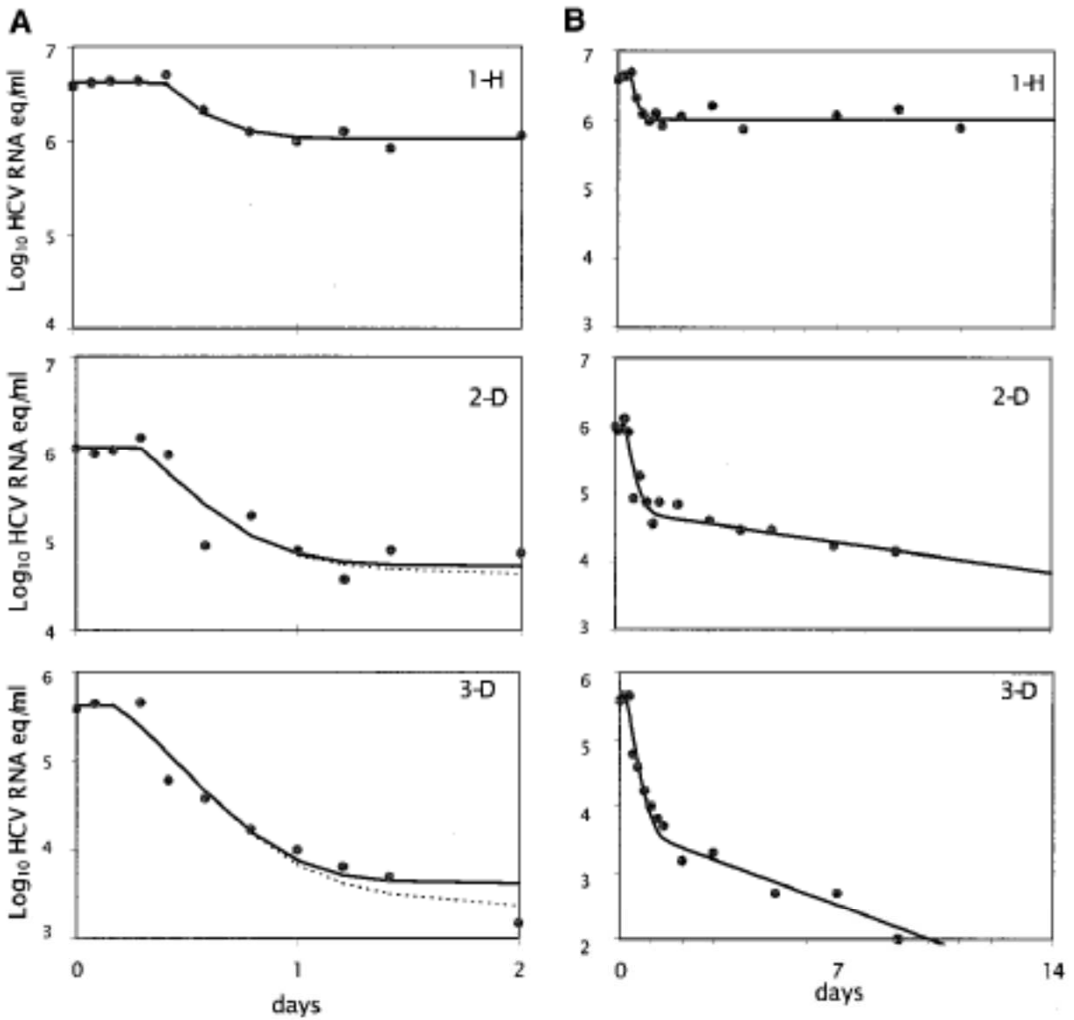


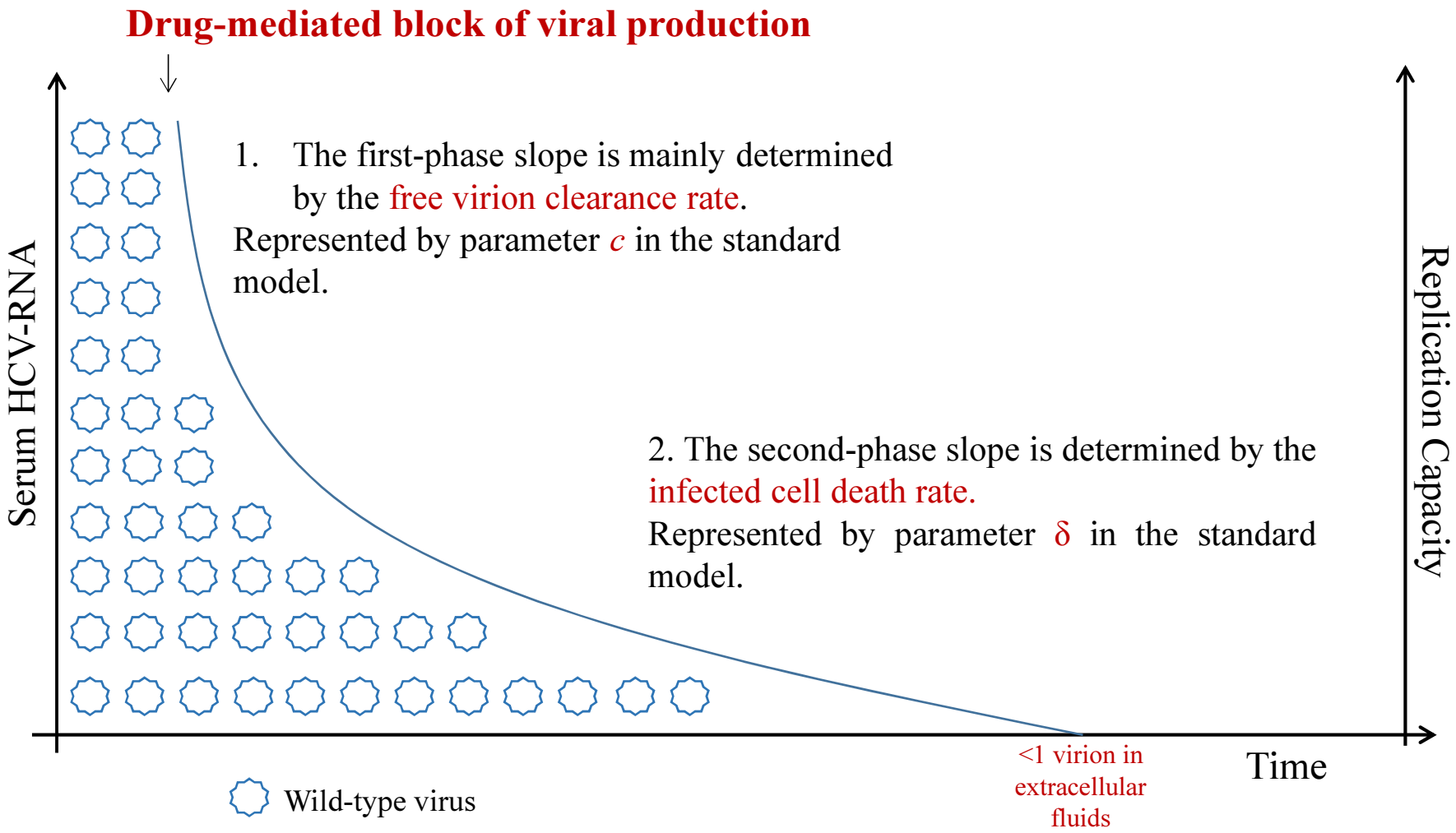
Fig. 1. HCV kinetics during the first 2 days (**A**) and the first 14 days (**B**) of IFN therapy for three representative patients receiving three different daily doses: 5 mIU (first row), 10 mIU (second row), and 15 mIU (third row). A biphasic viral decline can be observed. The first-phase slope is mainly determined by the free virion clearance rate and therapy efficacy. The second-phase slope is determined by the infected cell death rate and the efficacy and has large interpatient variation. The ratio between the viral load at day 2 and at day 0 gives a good estimate of the antiviral efficacy. On average, the slope is faster and the decrease is larger for the two higher doses (see Table 1). Solid lines in (A) are the best fit of the model to the viral load data (circles) assuming a constant level of infected cells (Eq. 4). Solid lines in (B) and dotted lines in (A) show the best fit with the full solution of the model (Eq. 5). Parameter values used are given in Table 1.

With IFN: biphasic kinetics of HCV-RNA decline

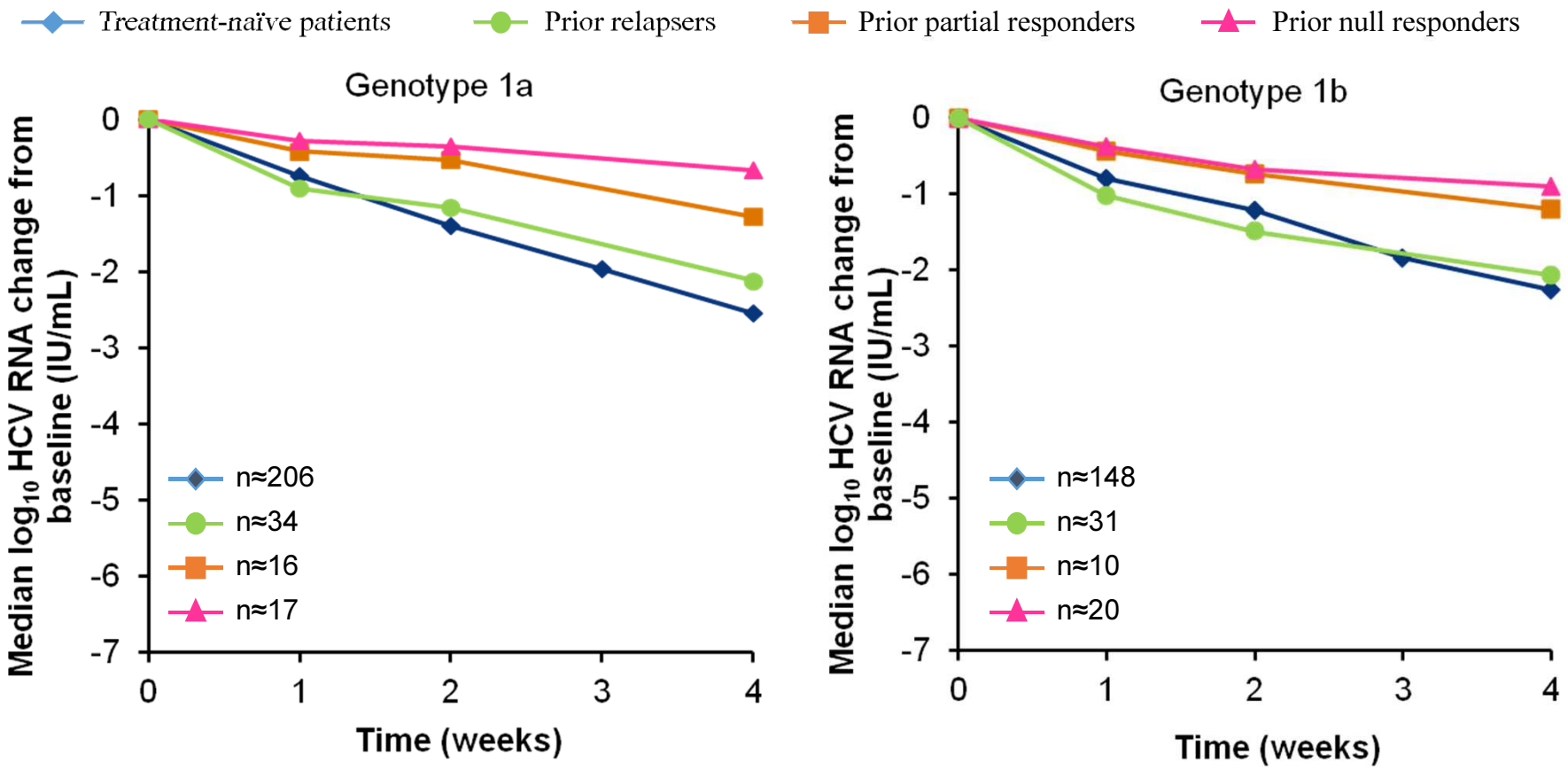
The virion clearance rate was, on average, $c = 6.2 \pm 1.8$ days^{-1}, corresponding to an average half-life of $t_{1/2} = 2.7$ hours (range, 1.5 to 4.6 hours) for free serum virions.

Neumann AU et al., Science 1998

Biphasic HCV-RNA decline following anti-HCV treatment according to the standard model of HCV kinetics



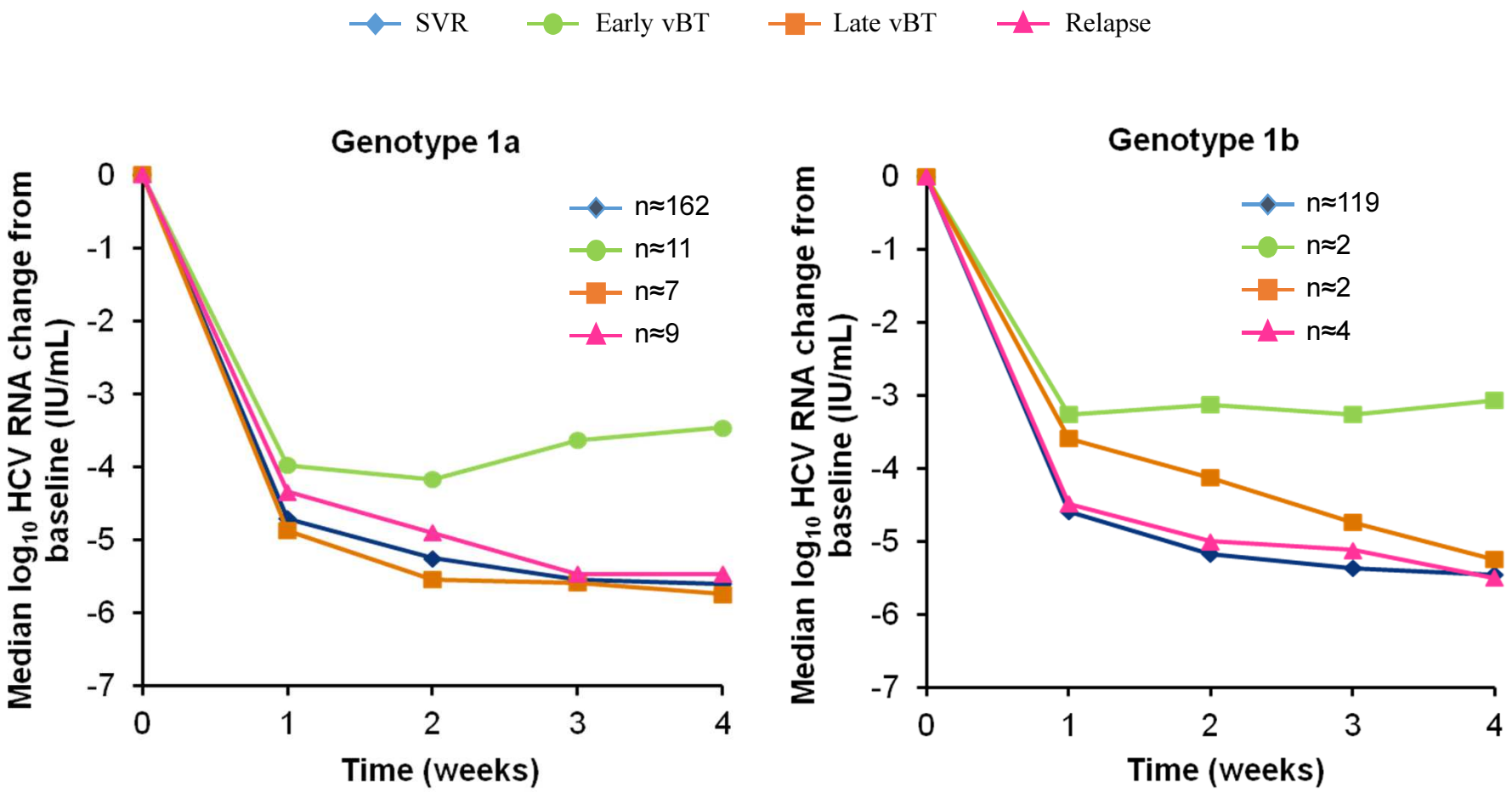
Median HCV RNA Change From Baseline for First 4 Weeks of pegIFN+Riba Treatment



Picchio G, presented at EASL 2012

Treatment-naïve patients were from ADVANCE; treatment-experienced patients were from REALIZE; all patients were in PR48 (control) arms

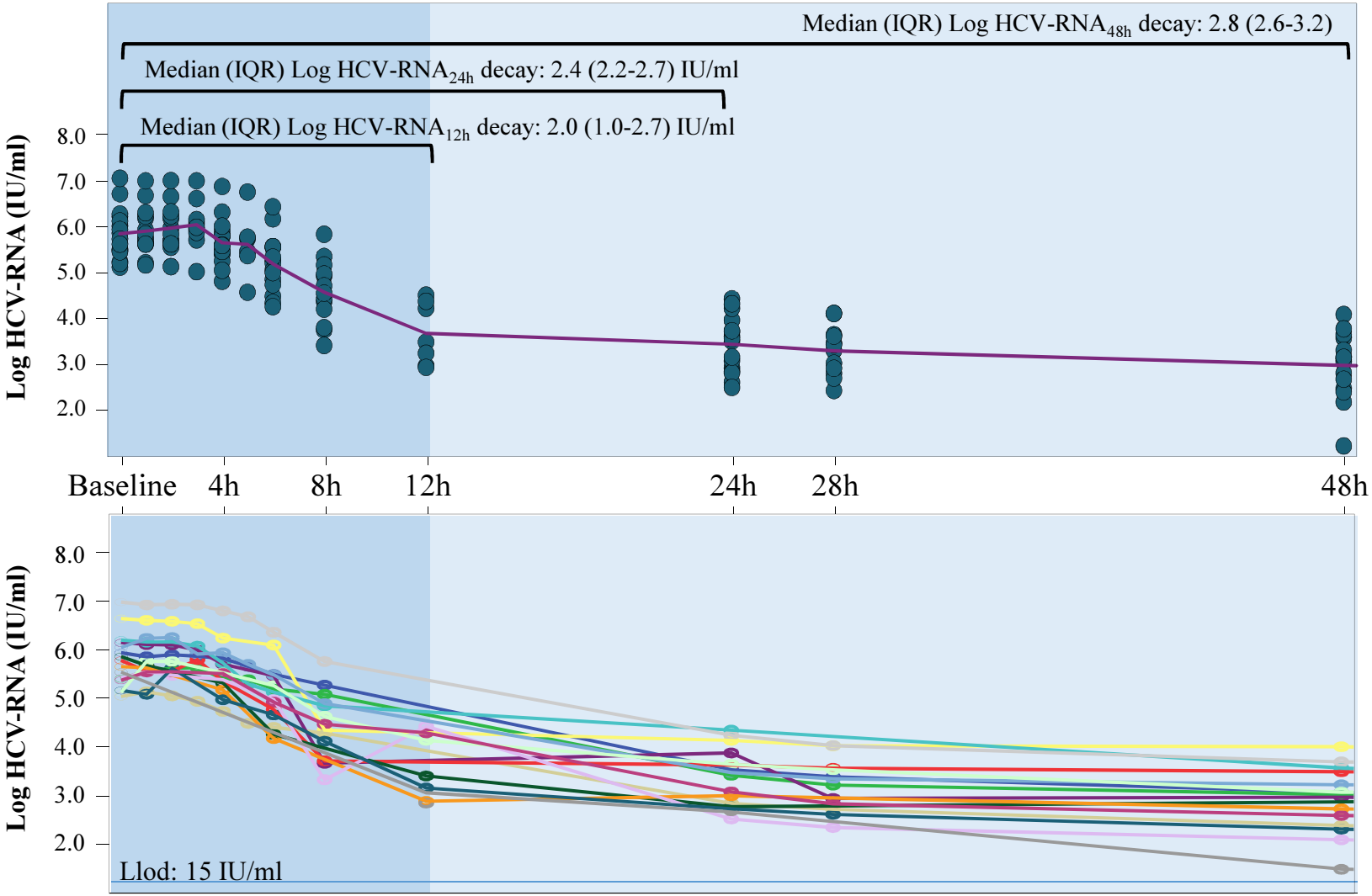
Median HCV-RNA Change From Baseline for First 4 Weeks of Telaprevir+pegIFN+Riba: Treatment-naïve Patients



Picchio G, presented at EASL 2012

Treatment-naïve patients were from ADVANCE (T12/PR)

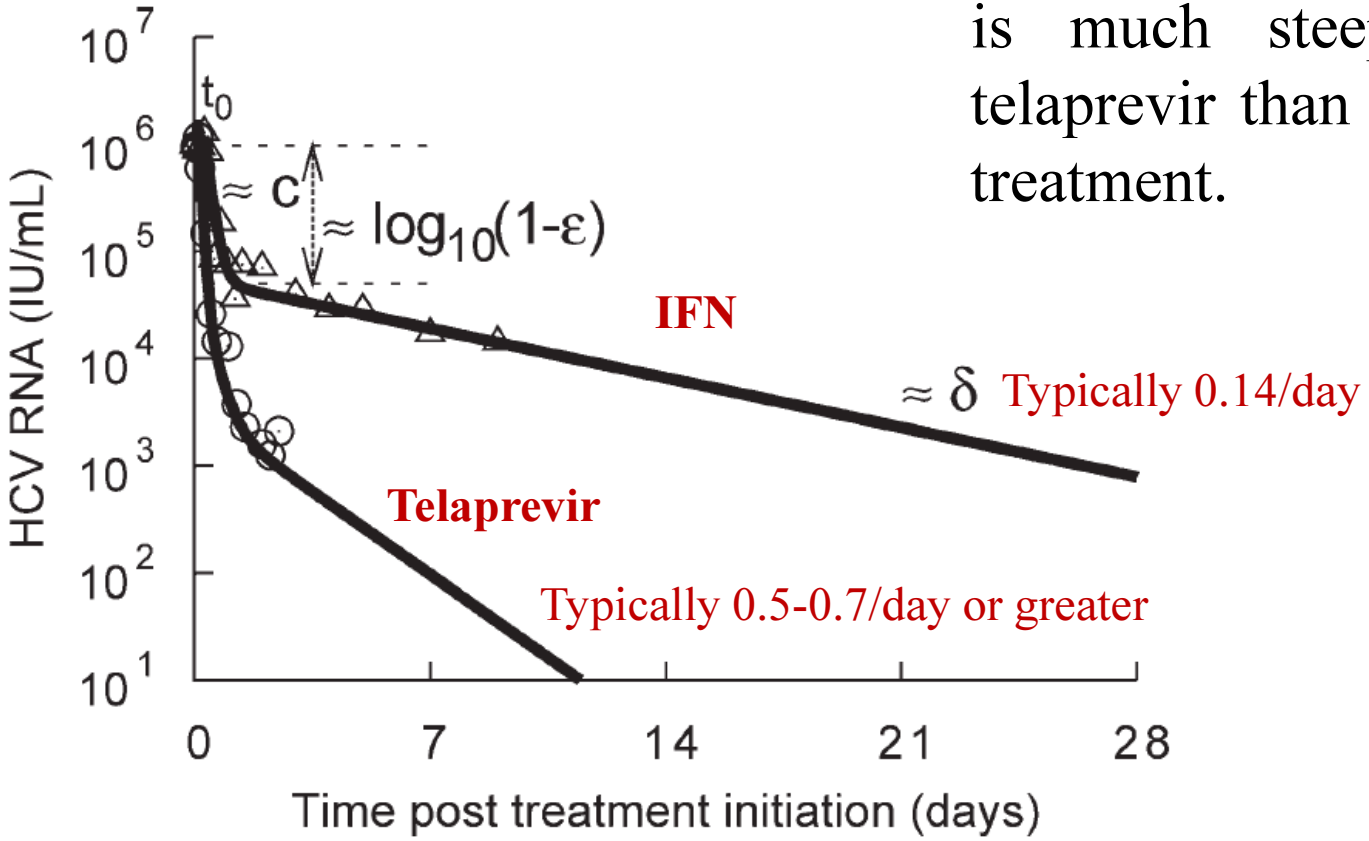
Both phases of HCV-RNA decline can be already visualized within the first 48 hours of telaprevir-based triple therapy



Cento V et al, Dig Liv Dis 2015

IFN vs HCV protease inhibitor (telaprevir): 2nd phase decline

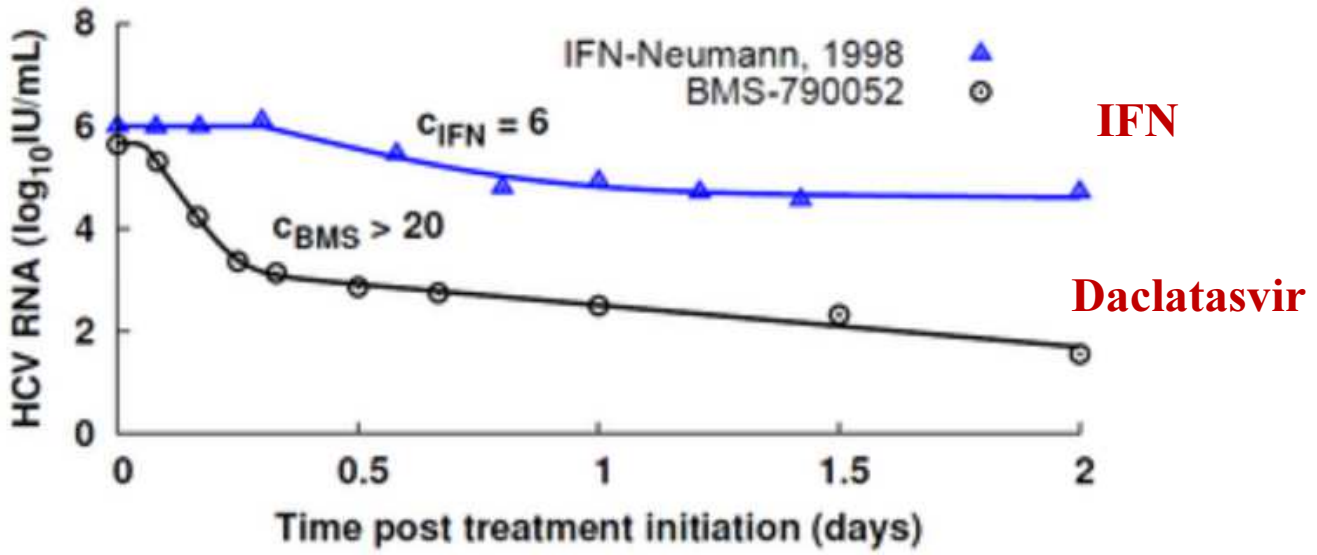
The second-phase slope is much steeper with telaprevir than with IFN treatment.



Guedj J and Perelson AS, Hepatology 2011

IFN vs HCV NS5A inhibitor (daclatasvir): 1st phase decline

The first-phase slope is much steeper with daclatasvir than with IFN treatment.



- The initial rate of viral decline is much more rapid
- Tends to be biphasic in most patients

Estimated rate of virus clearance, c, appears to change with drug

- IFN-therapy $c \sim 6\text{-}9\ d^{-1}$
- daclatasvir $c > 20\ d^{-1}$ (NS5A inhibitor)

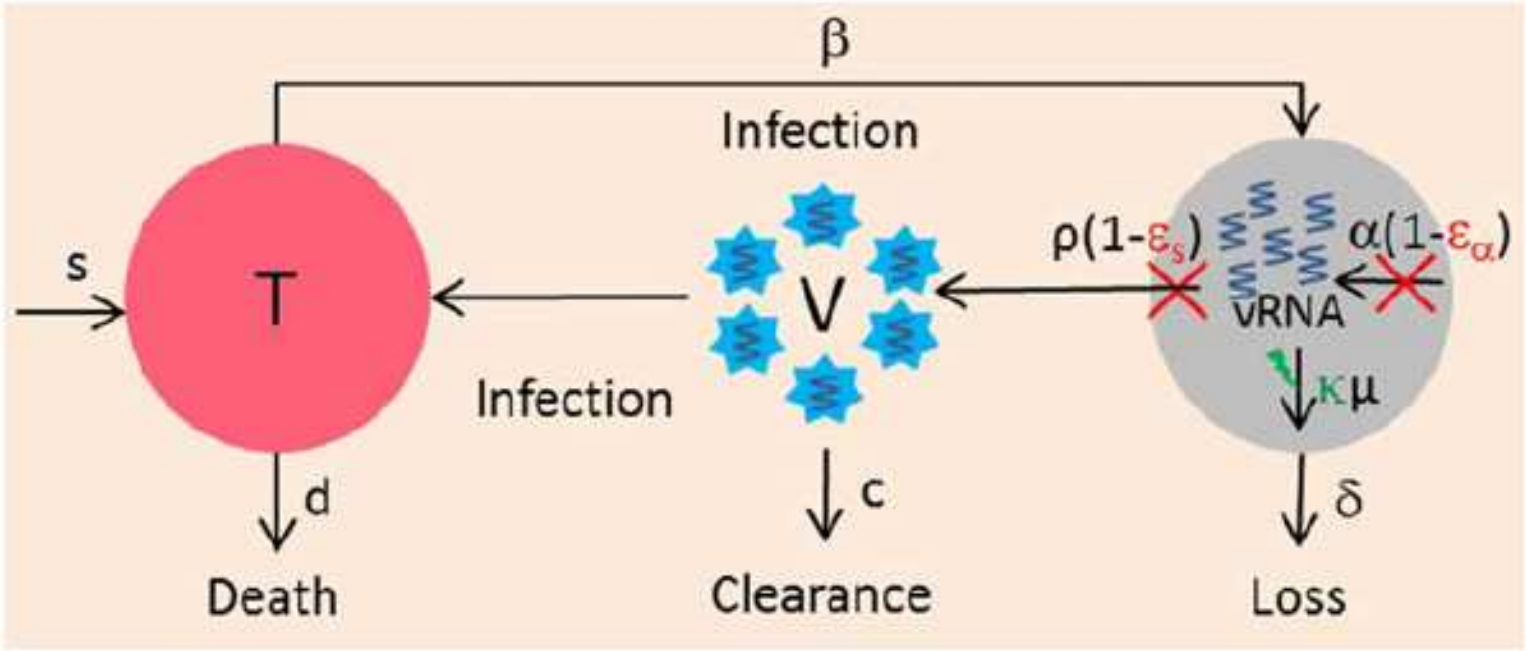
Standard models can not account for this.

Models that take into consideration intracellular events of viral replication and secretion can.

Idea, daclatasvir block both viral replication and secretion while IFN mainly blocks replication.

Multiscale model of HCV infection

The model predicts that the more rapid second phase obtained with DAAs could be due to the progressive eradication of intracellular viral content within infected cells.



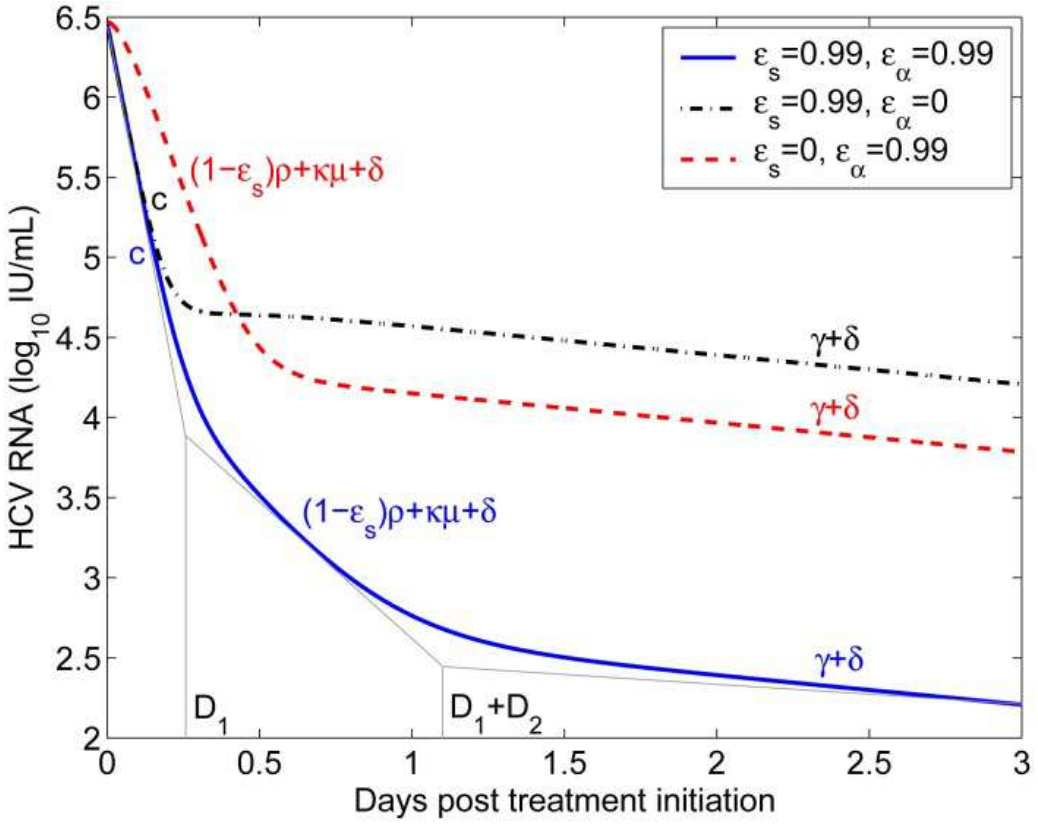
Nguyen THT and Guedj J, CTP Pharmacometrics Syst. Pharmacol. 2015

DAAs combinations directly interfere with **intracellular kinetics of vRNA** and have the ability to "**cure**" the infected hepatocytes

Newer DAAs have the ability to:

1. Block intracellular vRNA production.
2. Block viral assembly/secretion.
3. Enhance intracellular vRNA degradation.

As a consequence of multiple actions on HCV-life cycle, viral load declines in three phases



Conventional phase 1 now has 2 sub-phases.

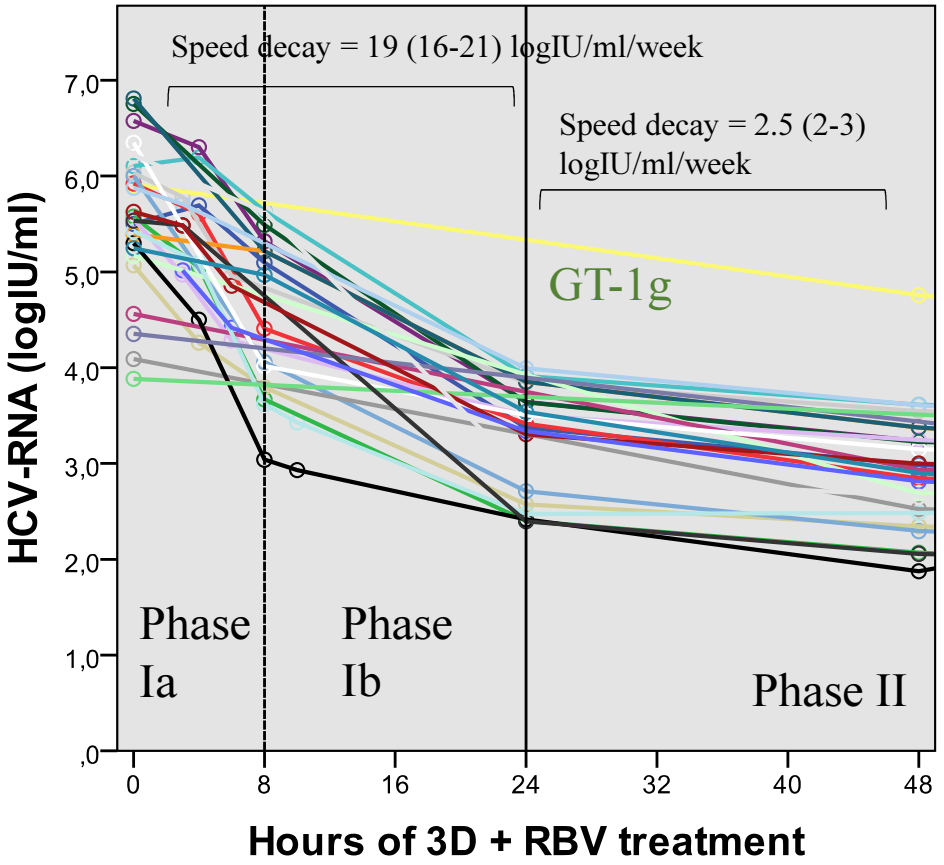
1. **Phase 1a**: that reflects virion clearance.
2. **Phase 1b**: that reflects the loss of intracellular vRNA due to encapsidation/secretion, degradation and loss of infected cells.

Rong et al. PLoS Comput Biol. 2013

Three-phase decline of HCV-RNA during 3D+RBV treatment in cirrhotic GT-1 patients

In the majority of patients, a **phase Ia** HCV-RNA decline was observed: HCV-RNA decay was > 1log IU/ml in 68.4% of patients (median [IQR] = 1.3 [0.6-1.9] logIU/ml) after only **8h of treatment**.

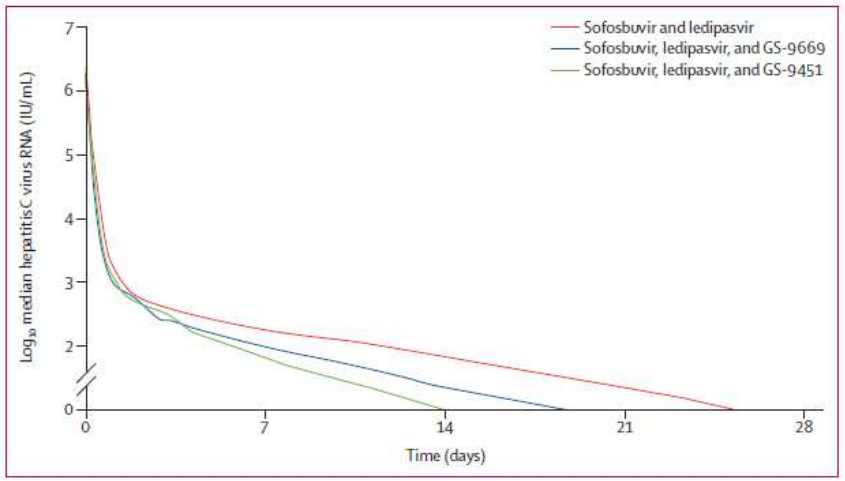
IU, international units; ALT, alanine transaminase; 3D, paritaprevir/r-ombitasvir-dasabuvir; RBV, ribavirin

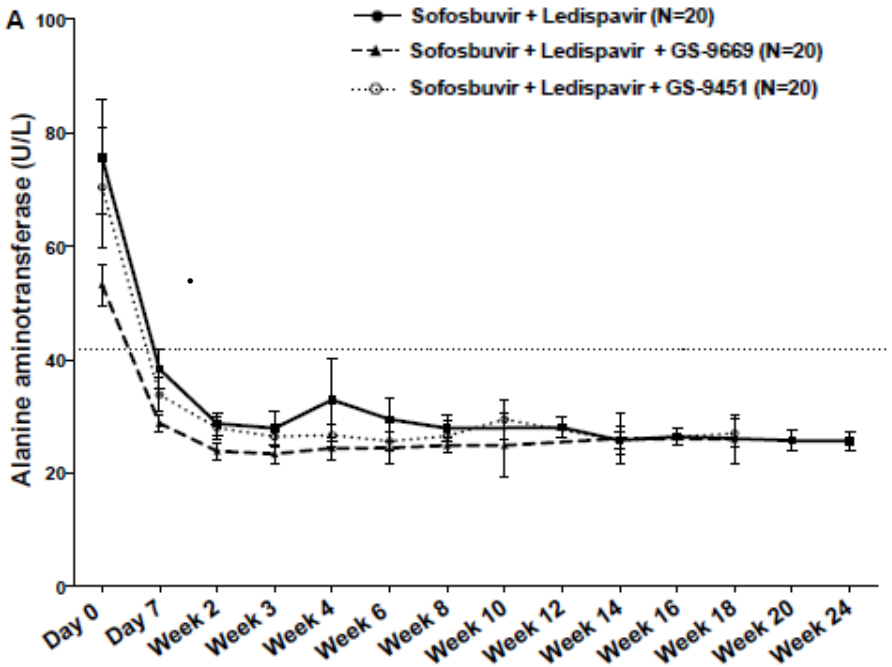


Cento V et al., AASLD 2015

The process of «**cell cure**» during all-oral treatment contributes to a sharp decline in **ALT** values

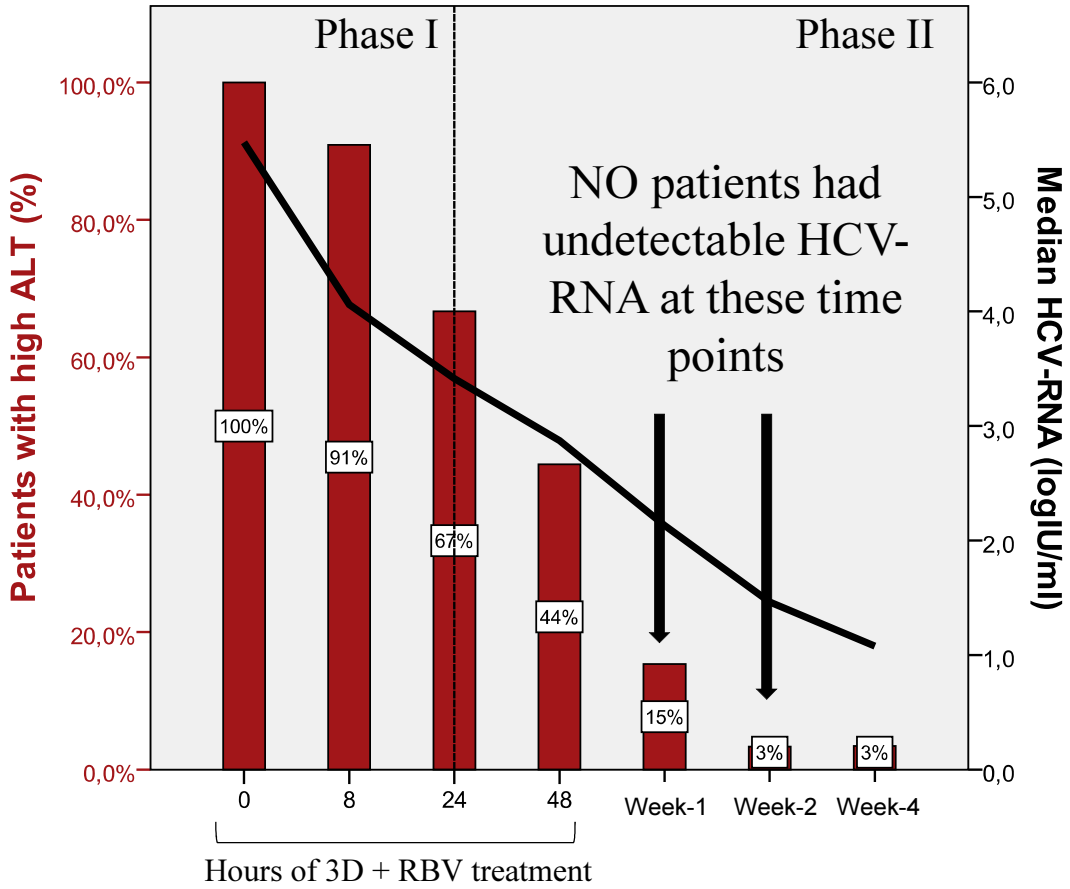
Decline in median **hepatitis C viral load** in drug-naïve, non-cirrhotic, GT-1 infected patients treated with all-oral DAAs combinations.





Kohli A et al., Lancet 2015

ALT decay was observed in all 3D treated patients during second-phase HCV kinetics, independent from HCV-RNA decline



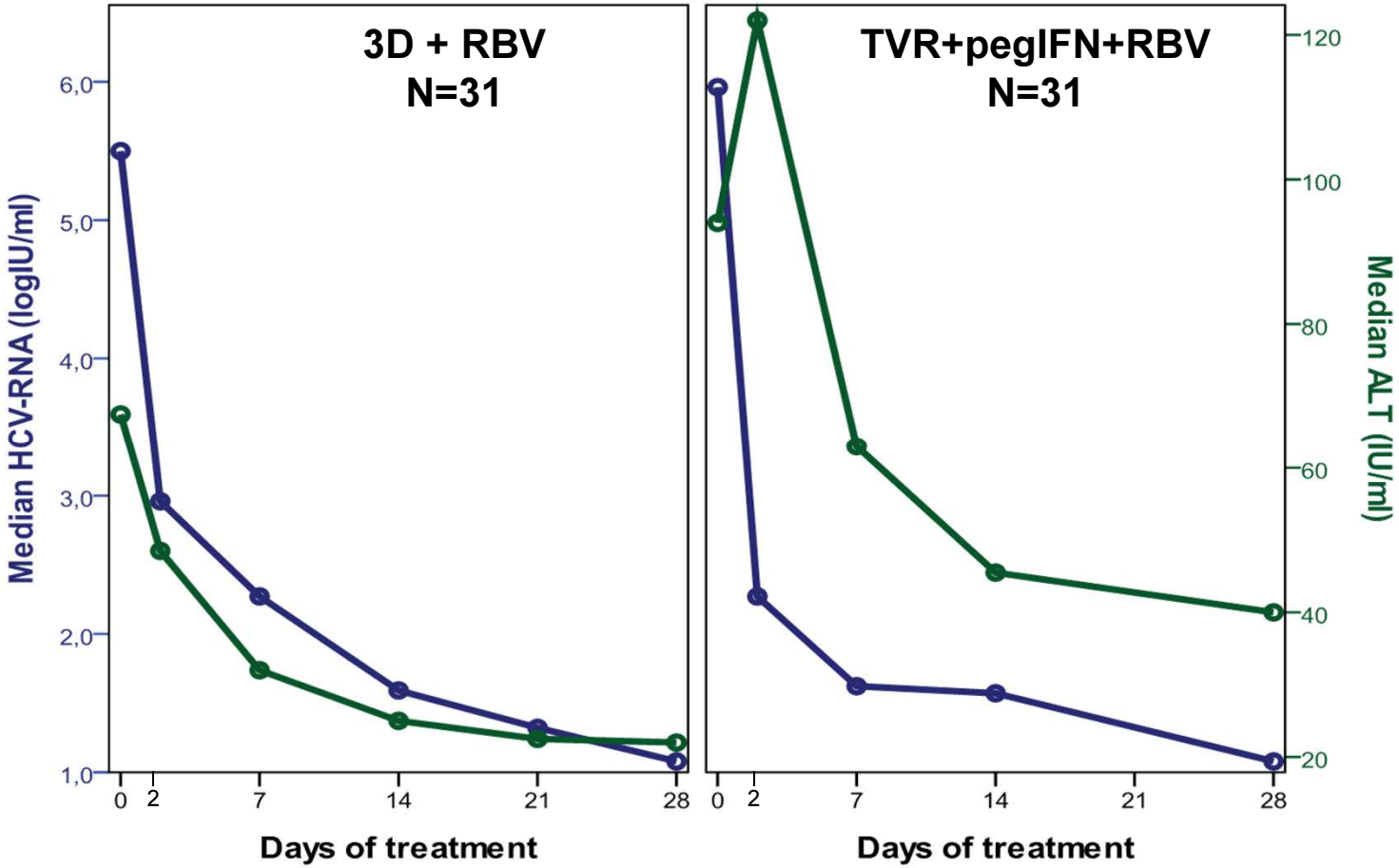
The median (IQR) time for ALT normalization was of 7 (3-8) days, corresponding to an HCV-RNA value of 266 (47-915) IU/ml.

No correlation was found among HCV-RNA decay at week-1 or week-2 and the corresponding ALT decay (rho = 0.042, p=0.812 and rho = 0.045, p=0.798 by Spearman test, respectively).

Only patients who started with ALT values above reference value are reported (88% of the total population). High ALT were defined as > 55 IU/ml in males and 45 IU/ml in females. IU, international units; ALT, alanine transaminase; 3D, paritaprevir/r-ombitasvir-dasabuvir; RBV, ribavirin

Cento V et al., AASLD 2015

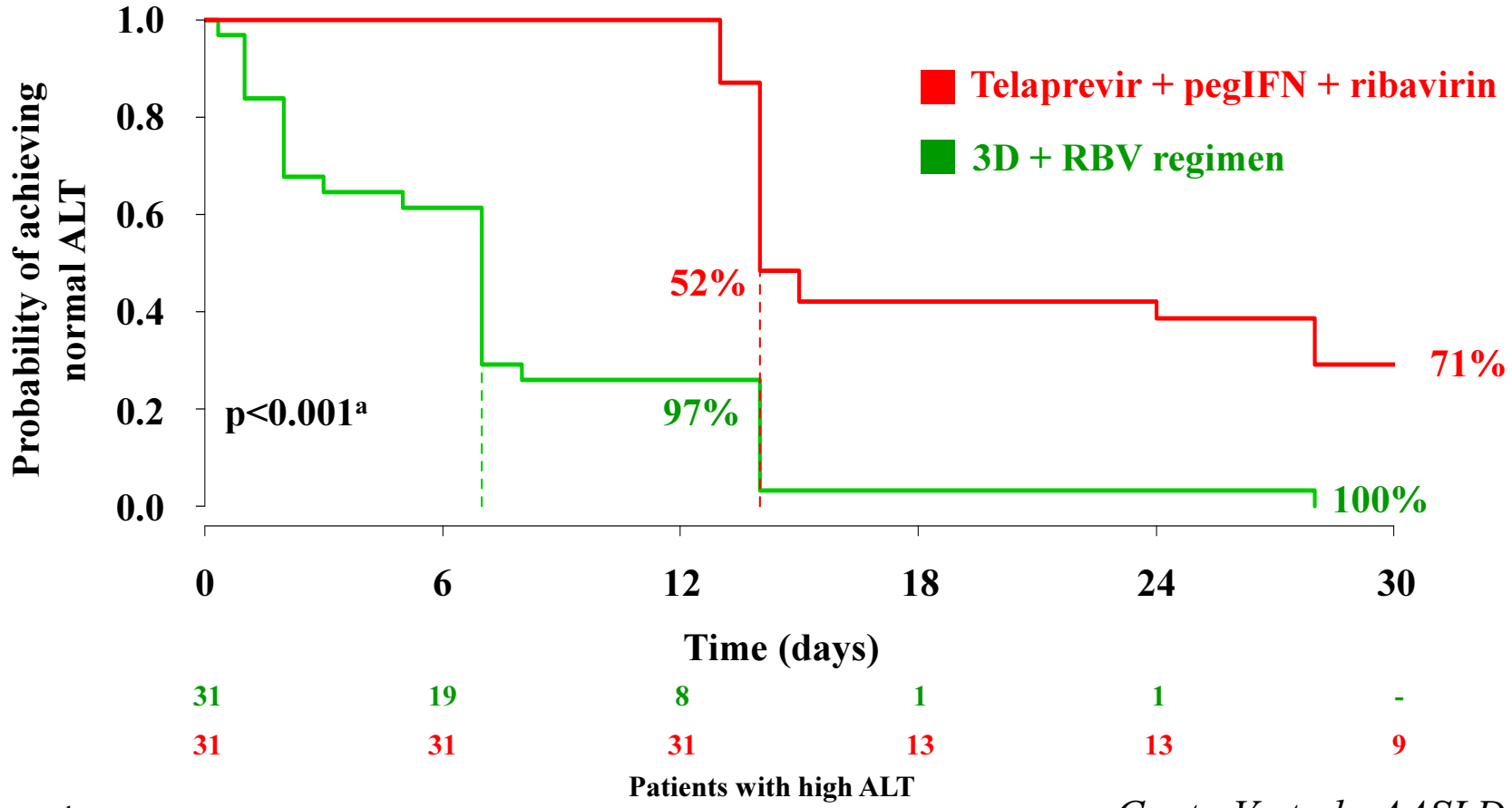
Aminotransferases kinetic differs among IFN-free/IFN including regimens



IU, international units; 3D, paritaprevir/r-ombitasvir-dasabuvir; RBV, ribavirin; TVR, telaprevir; pegIFN, pegylated interferon

Cento V et al., AASLD 2015

Patients treated with 3D + RBV regimen have 100% probability of normalizing ALT values within 4 weeks since treatment start, vs. 71% in patients receiving TVR+pegIFN (p<0.001)

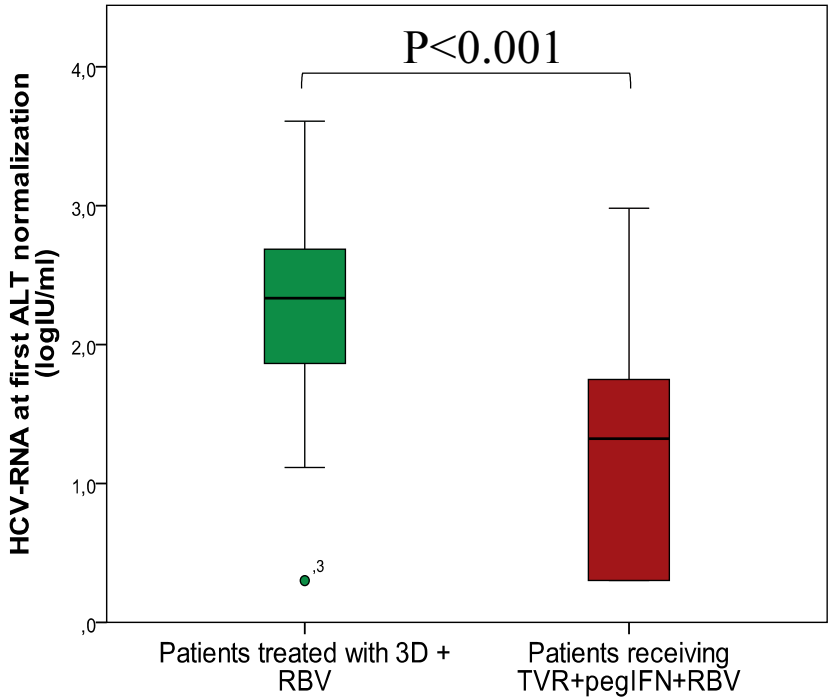


[a] By log-rank test.

Cento V et al., AASLD 2015

In all-oral treated-patients, ALT normalization precedes HCV-RNA clearance

The median (IQR) HCV-RNA associated with ALT normalization was **266 (47-915) IU/ml** in patients treated with **3D + RBV regimen**, much higher respecting to patients receiving **IFN**, in whom HCV-RNA had to reach values <LLOQ or <LLOD before observing ALT normalization (median [IQR] = **15 [TND-49] IU/ml**) (**P<0.001**).

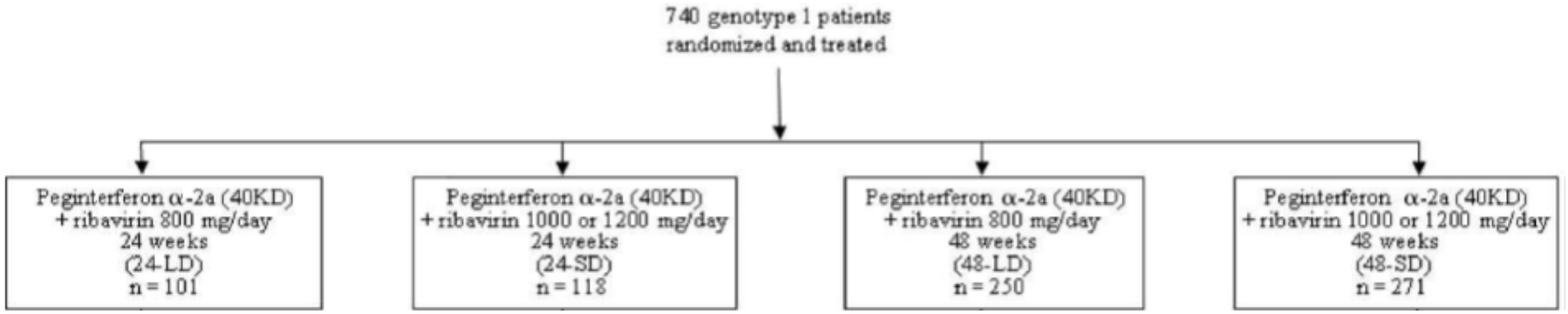


Normal ALT were defined as < 55 IU/ml in males and < 45 IU/ml in females; IU, international units; ALT, alanine transaminase; 3D, paritaprevir/r-ombitasvir-dasabuvir; RBV, ribavirin; TVR, telaprevir; pegIFN, pegylated interferon

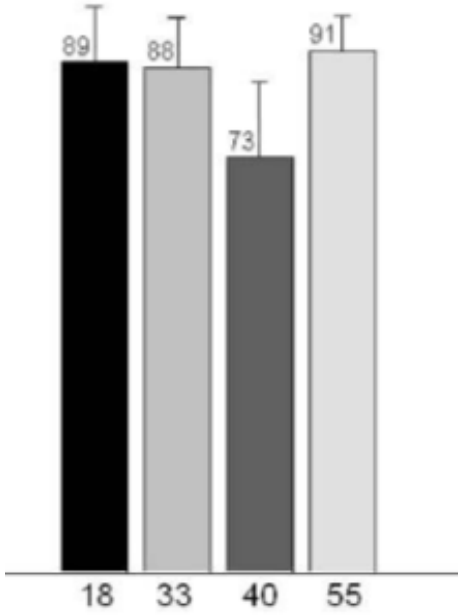
Cento V et al., AASLD 2015

Can we use viral kinetics in our daily clinical practice?

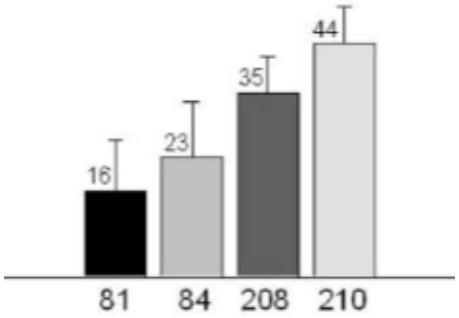
Historically, HCV-RNA levels have been a predictive on-therapy marker of treatment outcome



Patients with a rapid virological response at week-4



Patients without a rapid virological response at week-4



Jensen, Hepatology 2006

A level of HCV-RNA ≥ 12 IU/ml by week 4 of SOF treatment was a predictor for treatment failure in genotype 1 patients

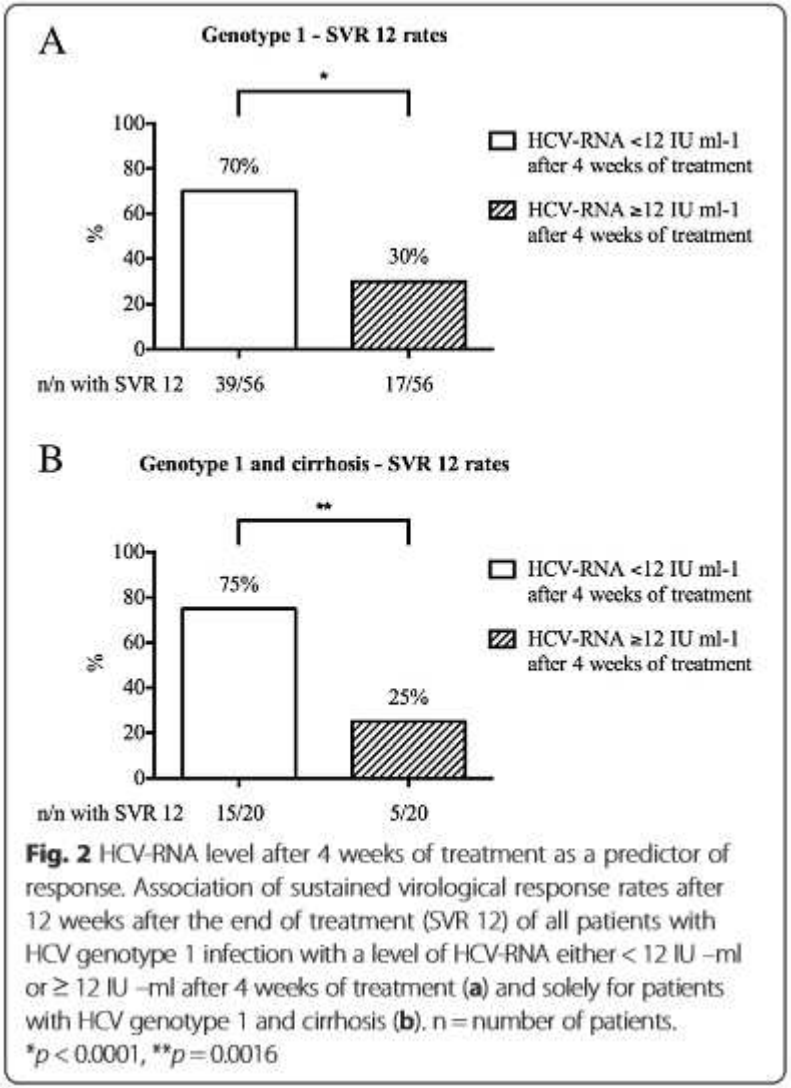


Fig. 2 HCV-RNA level after 4 weeks of treatment as a predictor of response. Association of sustained virological response rates after 12 weeks after the end of treatment (SVR 12) of all patients with HCV genotype 1 infection with a level of HCV-RNA either < 12 IU –ml or ≥ 12 IU –ml after 4 weeks of treatment (**a**) and solely for patients with HCV genotype 1 and cirrhosis (**b**). n = number of patients. *$p < 0.0001$, **$p = 0.0016$

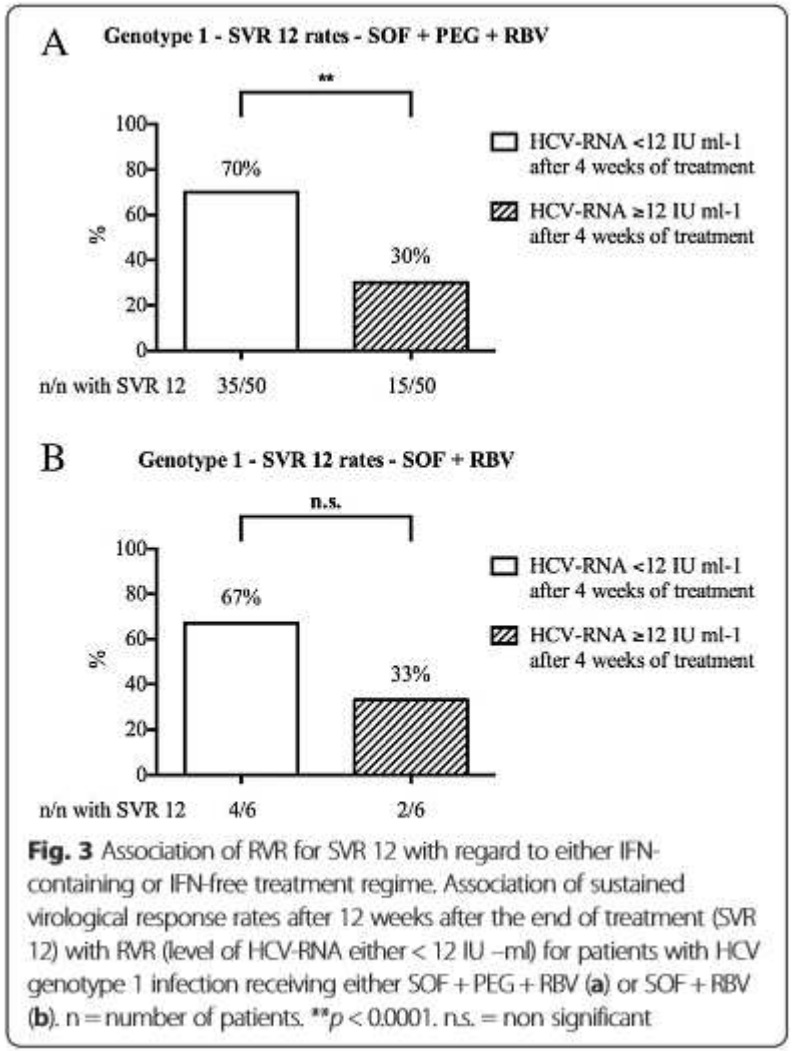


Fig. 3 Association of RVR for SVR 12 with regard to either IFN-containing or IFN-free treatment regime. Association of sustained virological response rates after 12 weeks after the end of treatment (SVR 12) with RVR (level of HCV-RNA either < 12 IU –ml) for patients with HCV genotype 1 infection receiving either SOF + PEG + RBV (**a**) or SOF + RBV (**b**). n = number of patients. **$p < 0.0001$. n.s. = non significant

Steinebrunner N et al., BMC Gastro 2015

Utility of Hepatitis C Viral Load Monitoring on Direct-Acting Antiviral Therapy

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[1]Critical Care Medicine Department, National Institutes of Health Clinical Center, National Institutes of Health, Bethesda, [2]Institute of Human Virology, University of Maryland, Baltimore, [3]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; and [4]Gilead Sciences Inc, Foster City, California

Background. Hepatitis C virus (HCV) RNA loads serve as predictors of treatment response during interferon-based therapy. We evaluated the predictive ability of HCV RNA levels at end of treatment (EOT) for sustained virologic response (SVR_{12}) during interferon-sparing direct-acting antiviral therapies.

Methods. HCV genotype 1–infected, treatment-naive patients were treated with sofosbuvir and ribavirin for 24 weeks (n = 55), sofosbuvir and ledipasvir for 12 weeks (n = 20), sofosbuvir, ledipasvir, and GS-9669 for 6 weeks (n = 20), or sofosbuvir, ledipasvir, and GS-9451 for 6 weeks (n = 19). Measurements of HCV RNA were performed using the Roche COBAS TaqMan HCV test and the Abbott RealTime HCV assay. Positive predictive value (PPV) and negative predictive value (NPV) of HCV RNA less than the lower limit of quantification (<LLOQ) at EOT for SVR_{12} were calculated.

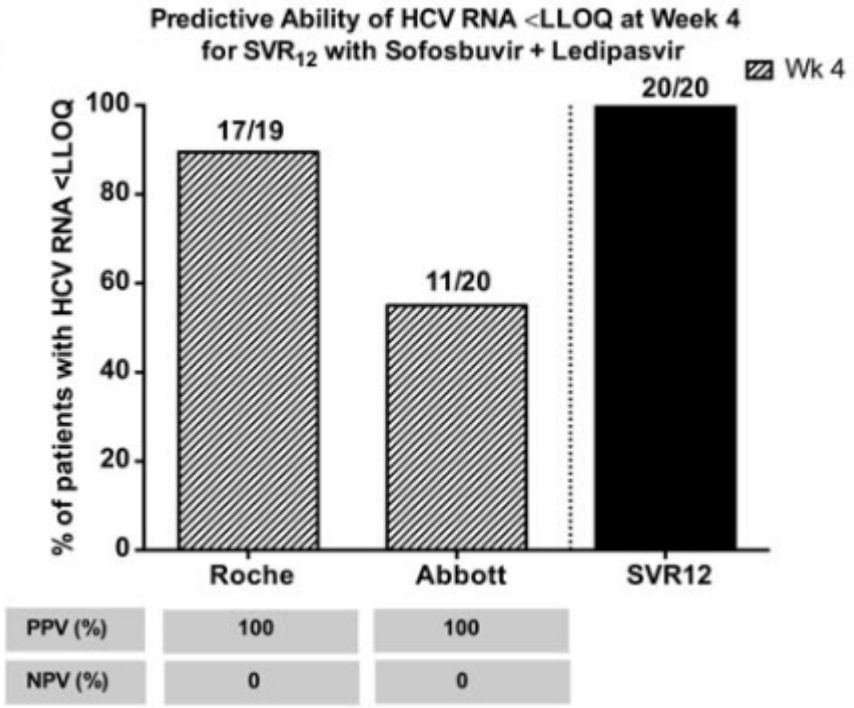
Results. All 55 patients treated with sofosbuvir and ribavirin had HCV RNA <LLOQ at EOT by the Roche and Abbott assays, but only 38 achieved SVR_{12} (PPV, 69%). Among patients treated with sofosbuvir and ledipasvir with or without GS-9669 or GS-9451, 100% (59/59) had HCV RNA <LLOQ by the Roche assay and 1 relapsed (PPV, 98%). By the Abbott assay, 90% (53/59) had HCV RNA <LLOQ, of whom 1 patient relapsed (PPV, 98%). Notably, 6 patients with HCV RNA ≥LLOQ at EOT (range, 14–64 IU/mL) achieved SVR_{12} (NPV, 0%). Quantifiable HCV RNA (range, 15–57 IU/mL) was measured 2 weeks posttreatment in 4 individuals, and 4 weeks posttreatment in 1 patient (14 IU/mL).

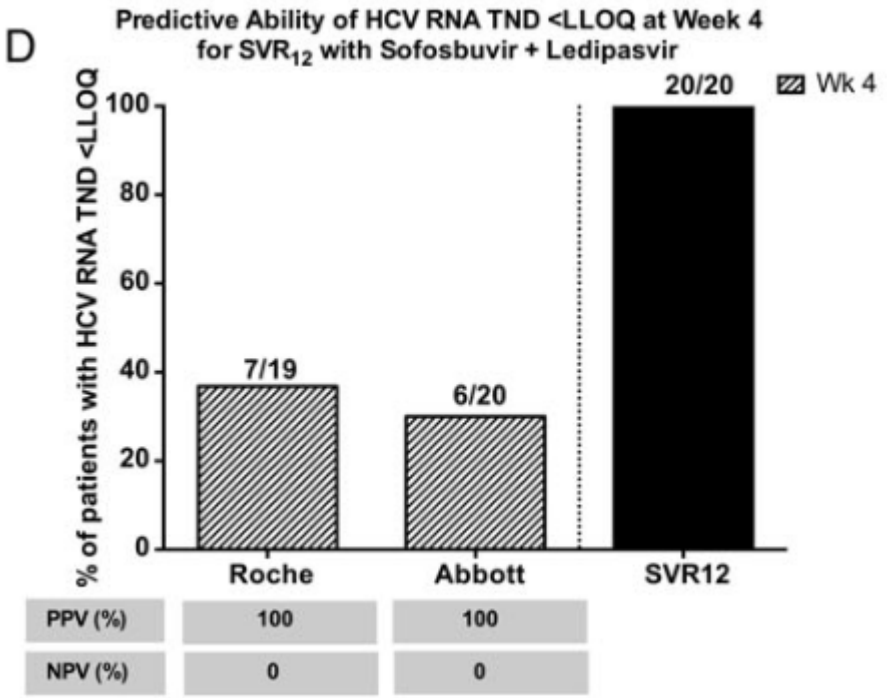
Conclusions. Contrary to past experience with interferon-containing treatments, low levels of quantifiable HCV RNA at EOT do not preclude treatment success.

Keywords. viral load; direct-acting antiviral; HCV RNA; hepatitis C.

HCV RNA measurements on treatment were not clinically useful for predicting SVR 12 in sofosbuvir-containing, interferon-free regimens

Low NPVs for HCV RNA at week 4 in patients treated with 6–12 weeks of sofosbuvir and ledipasvir with or without GS-9669 or GS-9451 suggest that the majority of patients with quantifiable or detectable HCV RNA during treatment achieve SVR12.

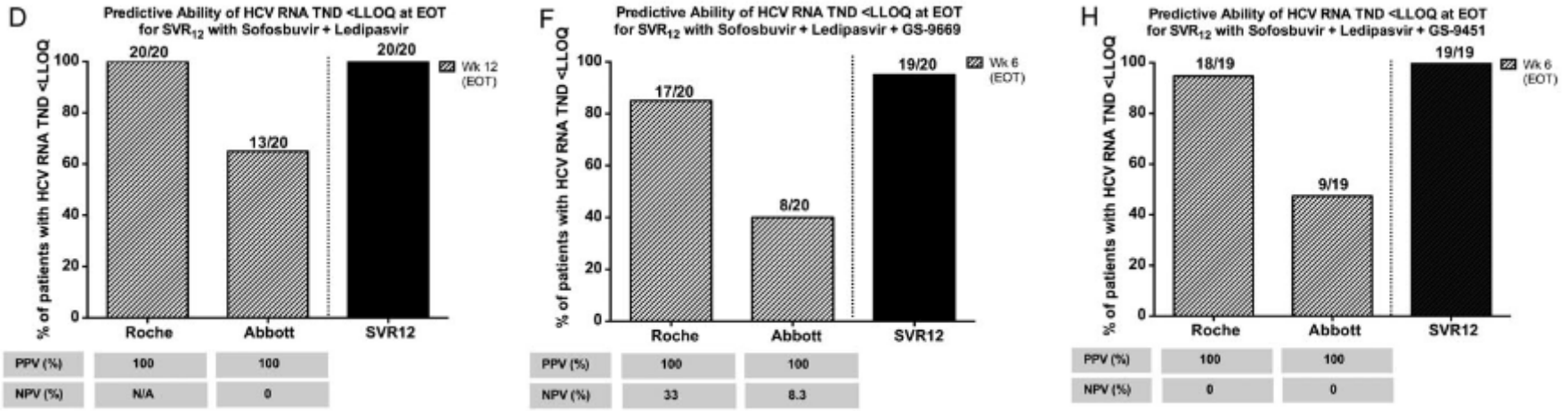




Sidharthan S et al., CID 2015

In contrast to what has been observed with interferon-containing therapy, low levels of quantifiable virus after treatment completion were not predictive of relapse with SOF-LED containing regimens

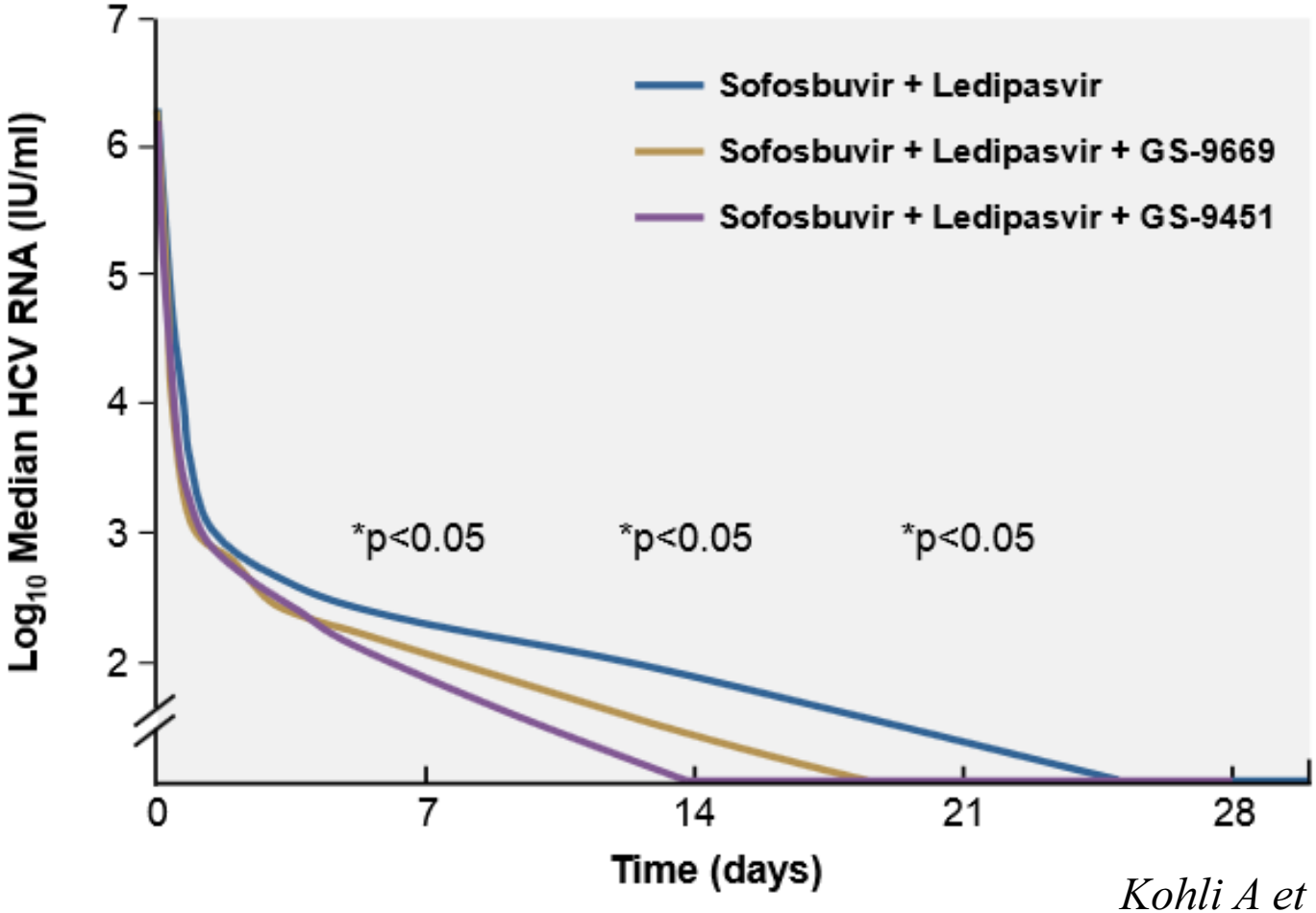
In the cohort of patients treated with 6 weeks of ledipasvir, sofosbuvir, and either GS-9669 or GS-9451, more than half had detectable HCV RNA at EOT by the Abbott assay, the majority of whom achieved SVR12.



What is the role of the host immune system?
Are we detecting noninfectious viral particles?

Sidharthan S et al., CID 2015

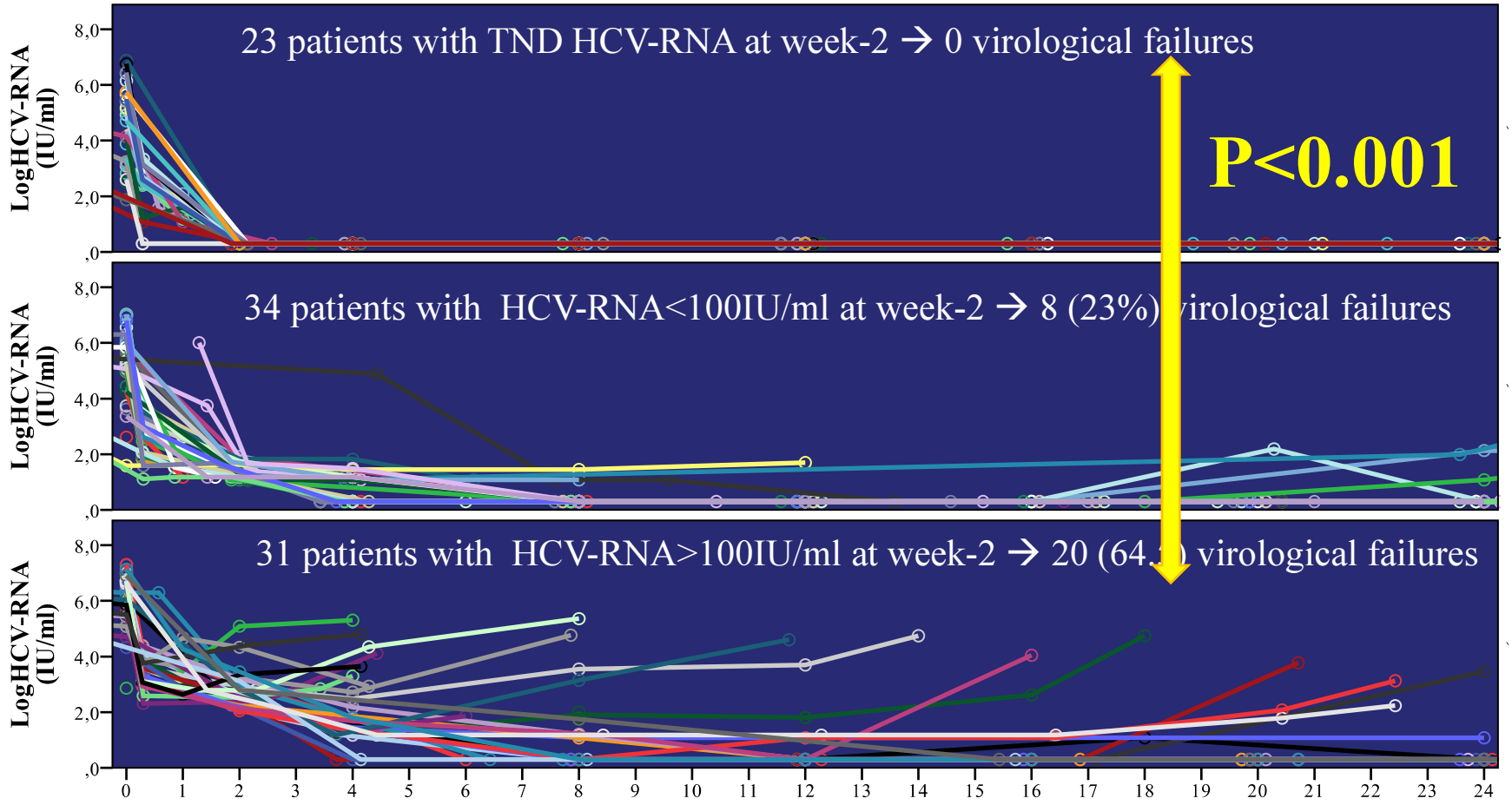
Since DAAs combinations induce a faster HCV-RNA decay … should we look at the more early phases of HCV-RNA kinetics?



Kohli A et al., Lancet 2015

2 weeks HCV-RNA values >100 IU/ml are associated with virological failure to first-generation PIs

After 2 weeks of PI administration, 23 patients were already TND and 35 had very low HCV-RNA levels (all <100 IU/ml), while 32 patients still had HCV-RNA higher than 2 log IU/ml (>100 IU/ml).



IU, international units; PI, protease inhibitor

Cento V et al., Dig Liv Dis 2015

In non-cirrhotic GT-1 patients treated with SOF+LED for 12 weeks, HCV-RNA levels at week-2 of treatment did not correlate with SVR12 rates

LEDIPASVIR + SOFOSBUVIR

Day 0	Wk 2	Wk 4	Wk 6	Wk 8	Wk 12	SVR2	SVR4	SVR8	SVR12
665 703	1704	833	316	85	TD	TD	TND	TND	TND
4 844 596	441	78	36	26	TD	TD	TND	TND	TND
3 067 577	188	80	46	15	TD	TND	TND	TND	TND
2 807 294	80	58	26	19	TD	TND	TND	TND	TND
1 851 819	34	TD	TD	TND	TD	TND	TND	TND	TND
4 844 596	258	93	30	15	TD	TND	TND	TND	TND
12 502 372	1086	101	15	TD	TD	TD	TND	TND	TND

Sidharthan S et al., CID 2015

Response-guided therapy using an all-oral regimen of 3 direct-acting antivirals cured a majority of easier-to-treat genotype 1b hepatitis C patients in just 3 weeks

The open-label SODAPI study (NCT02470858) included 26 Chinese patients with HCV subtype 1b, which is easier to treat than subtype 1a.

- Just over half had been previously treated for hepatitis C.
- Most had absent or mild liver fibrosis (stage F0-F1) and none had cirrhosis.
- About two-thirds had the favorable IL28B "CC" gene pattern associated with good interferon response.
- At baseline the mean HCV viral load was approximately 6.5 log IU/mL.

Lau GK et al., AASLD 2015. Abstract LB-32.

Response-guided therapy using an all-oral regimen of 3 direct-acting antivirals cured a majority of easier-to-treat genotype 1b hepatitis C patients in just 3 weeks

Participants were randomly assigned to receive 3-drug regimens consisting of the NS5B nucleotide polymerase inhibitor **sofosbuvir**, one of the NS5A replication complex inhibitors **ledipasvir** or **daclatasvir**, and one of the HCV NS3/4A protease inhibitors **asunaprevir** or **simeprevir**.

- Group 1: sofosbuvir/ledipasvir and asunaprevir (n=12);
- Group 2: sofosbuvir, daclatasvir, and simeprevir (n=6);
- Group 3: sofosbuvir, daclatasvir, and asunaprevir (n=8).

Participants with **rapid virological response** (**RVR**), defined as **HCV RNA <500 IU/mL by day 2**, stopped treatment after **3 weeks**, while the remainder continued for 8 to 12 weeks

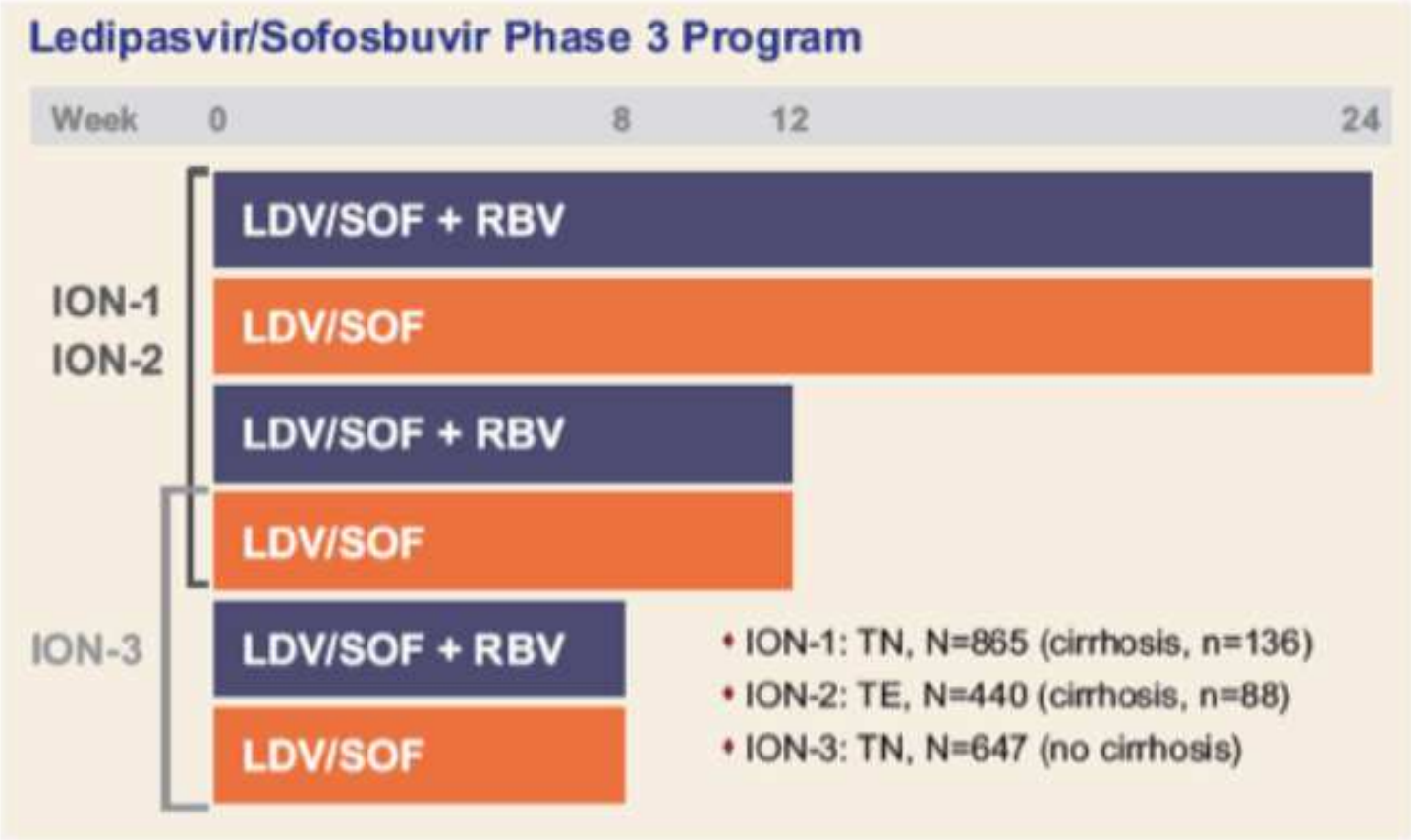
Lau GK et al., AASLD 2015. Abstract LB-32.

- Overall, 18 participants (67%) -- 6 in each treatment arm -- experienced RVR at day 2 of therapy. **All 18 people who had RVR at day 2 and completed treatment at 3 weeks achieved SVR12**.
- People who achieved RVR had *lower baseline viral load* on average. People with *prior treatment experience* and those with *more fibrosis* appeared somewhat less likely to achieve RVR, but the subgroup numbers were small and differences did not reach statistical significance.

Compared to standard treatment using sofosbuvir/ledipasvir for 8 weeks or sofosbuvir plus daclatasvir for 12 weeks, the researchers calculated that the 3-week regimen could save between $37,454 and $107,045 per patient.

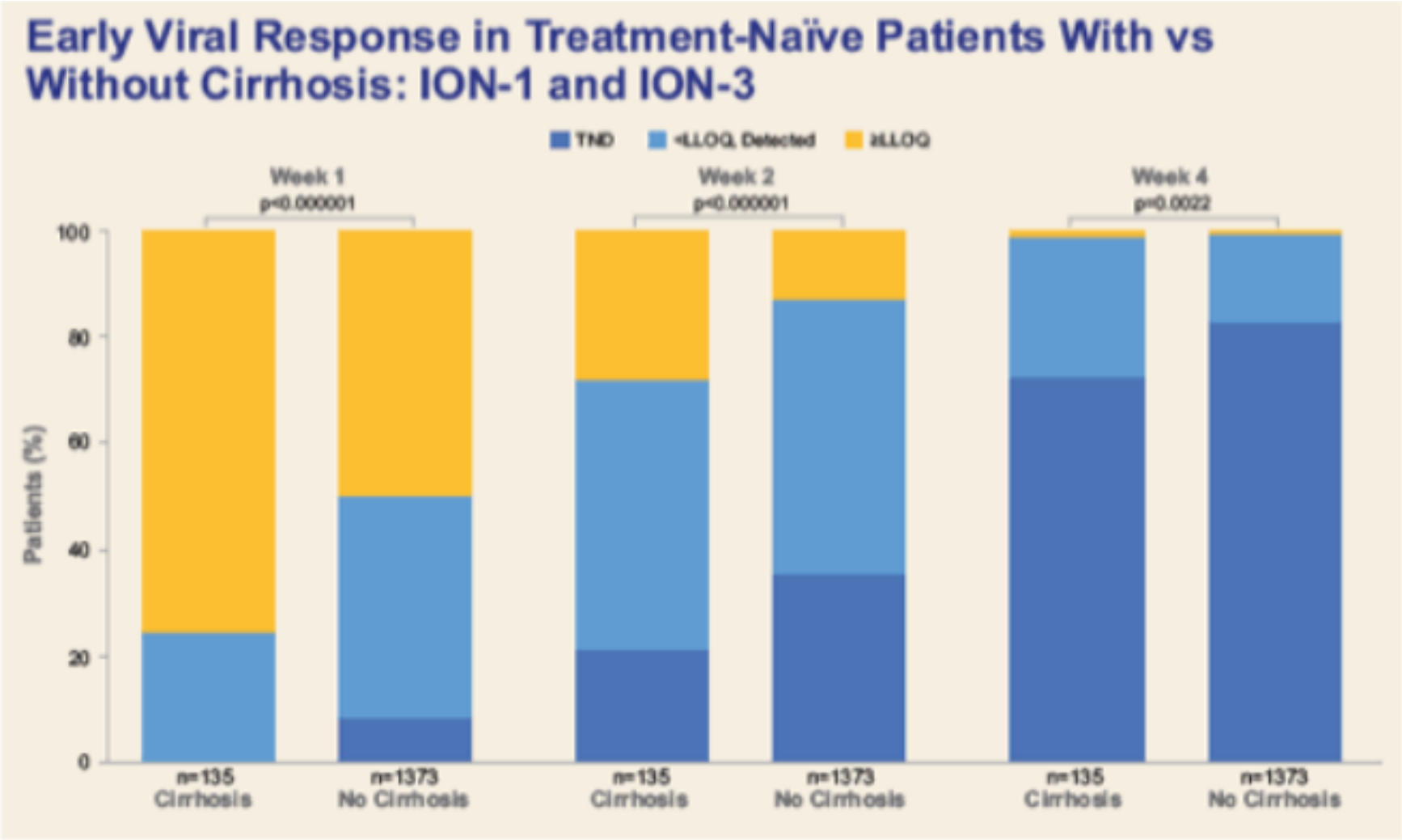
Lau GK et al., AASLD 2015. Abstract LB-32.

On-Treatment HCV RNA as a Predictor of Virologic Response in the Ledipasvir/Sofosbuvir Phase 3 Program for HCV Genotype 1 Infection: Analysis of the ION-1, ION-2, and ION-3 Studies



Welzel, AASLD 2014

Viral clearance on interferon-free and ribavirin-free regimes is slower in patients with cirrhosis than in patients without cirrhosis



Welzel, AASLD 2014

Overall, 9 patients with cirrhosis (2 in ION-1 and 7 in ION-2) relapsed ….

- Patients with cirrhosis who relapsed after 12 weeks of treatment had relatively high **baseline viral loads** and significantly higher **viral loads at week-1, 2 and 4** that did those with SVR12.
- In patients with cirrhosis and HCV-RNA > LLOQ at weeks 1 and 2, SVR12 rates were significantly higher after **24** vs. **12 weeks** of treatment (p=0.027 and p=0.025, respectively).

Positive and Negative Predictive Values for SVR12

		ION-1: TN, n/N (%)				ION-2: TE, n/N (%)			
		Platelet Count ≤138,500/µL		Platelet Count >138,500/µL		Platelet Count ≤138,500/µL		Platelet Count >138,500/µL	
		PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
Wk1	TND	0/1 (0)	1/63 (1.6)	0	0/71 (0)	2/2 (100)	2/46 (4)	1/2 (50)	4/38 (11)
	<LLOQ	18/19 (95)	1/45 (2)	15/15 (100)	0/56 (0)	12/12 (100)	2/36 (6)	4/5 (80)	4/35 (11)
Wk2	TND	14/15 (93)	1/49 (2)	14/14 (100)	0/57 (0)	15/15 (100)	2/33 (6)	4/5 (80)	4/35 (11)
	<LLOQ	47/48 (98)	1/16 (6)	49/49 (100)	0/22 (0)	39/40 (98)	1/8 (13)	17/18 (94)	4/22 (18)
Wk4	TND	49/50 (98)	1/14 (7)	48/48 (100)	0/23 (0)	39/41 (95)	0/7 (0)	26/27 (96)	4/13 (31)
	<LLOQ	62/64 (97)	0	70/70 (100)	0/1 (0)	45/47 (96)	0/1 (0)	35/39 (90)	1/1 (100)
Wk6	TND	60/62 (97)	0/2 (0)	67/67 (100)	0/4 (0)	44/46 (96)	0/2 (0)	32/36 (89)	1/4 (25)
	<LLOQ	62/64 (97)	0	71/71 (100)	0	46/48 (96)	0	35/40 (88)	0

Welzel, AASLD 2014

In cirrhotic patients, the selection of optimal treatment duration can be made following the principles of response-guided therapy

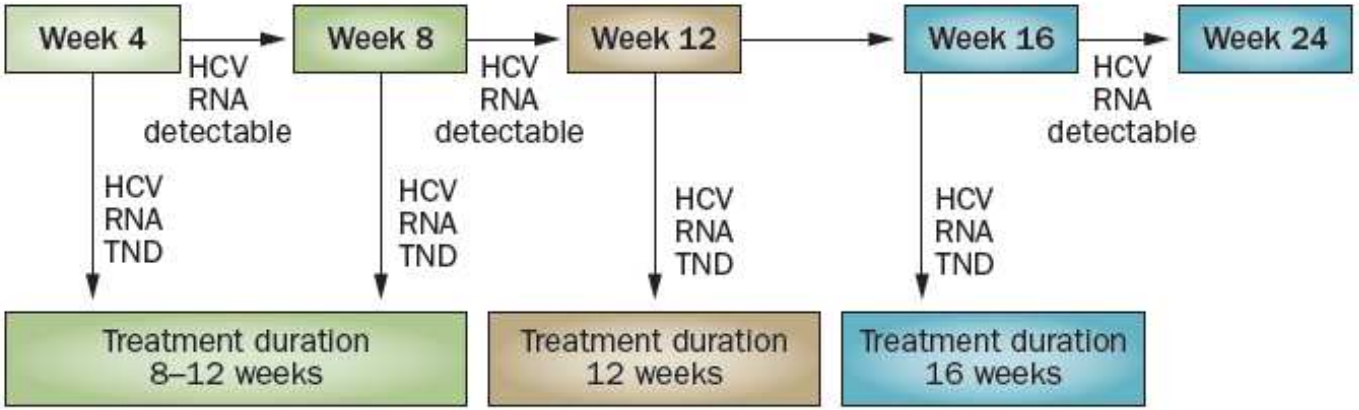


Figure 3 | Selection of optimal treatment duration in patients with cirrhosis. This proposed algorithm is based on interim data in which all patients with advanced liver diseases and undetectable HCV (and who completed 12 weeks follow-up) at week 8 had an SVR12.[29] TND by One Signal Amplification (Versant® HCV RNA 3.0, Siemens Corp. USA). The predictive value of HCV-RNA by One Signal Amplification (Versant® HCV RNA 3.0, ART) at week 4 was low in patients without cirrhosis.[80] Using this system, undetectable HCV RNA rates were lower than with the COBAS® AmpliPrep/Cobas TaqMan (Roche Molecular Systems Inc., USA).[80–82] Abbreviations: SVR12, sustained virologic response at 12 weeks; TND, target not detected.

Ferenci P, Nat Rev 2015

Conclusions I

- All-oral treatment administration in HCV infected patients leads to a fast and deep, triphasic HCV-RNA decay.
- A consistent and contextual fast and deep ALT decay is observed, thanks to DAAs' ability to cure the infected hepatocytes.
- Different from IFN-including regimen, whose antiviral activity also depend upon hepatocyte death, all-oral approaches have the ability to induce "cell-cure", without killing of infected cells. Therefore, ALT levels can become normal even when HCV-RNA clearance is not yet fully achieved.

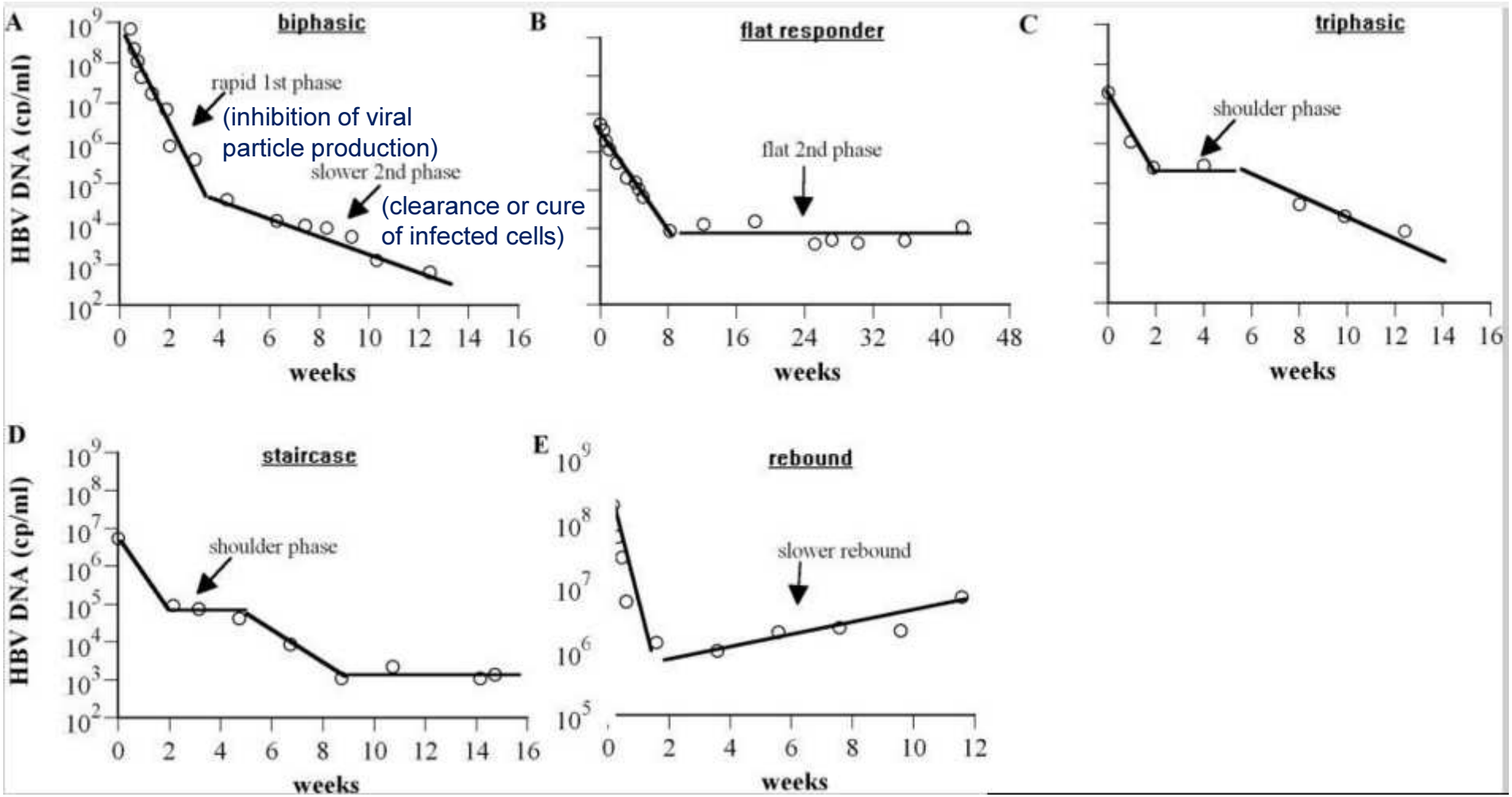
- Whether this can accelerate the time-to-improvement of liver architecture after curing HCV infection, is still matter of research.

Conclusions II

- In contrast with IFN-containing regimens, a Rapid Viral Response after 4 weeks of INF-free treatment seems not to correlate with SVR12.
- Since viral kinetics is faster in patients receiving multiple DAAs, maybe we should move backwards, looking for clinically useful HCV-RNA check-points.
  - Week-2 in PIs plus IFN triple regimens …. Data are missing on IFN-free regimens.
  - 48h in 3-DAAs combination regimens can selected easy to treat patients elegible for shorter treatments.
- Cirrhotic patients have a slower HCV-RNA kinetics, and in this population response guided therapy can still have a role in determining the optimal duration of treatment.

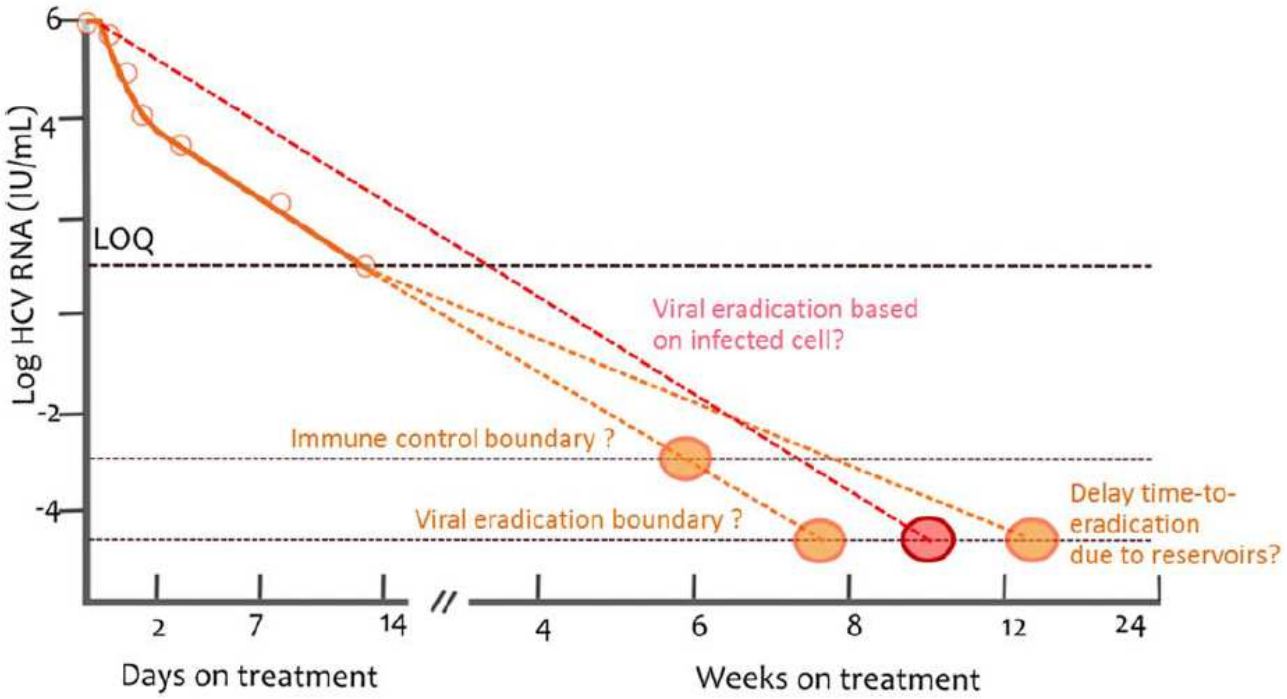
Heteregenous patterns of viremia decay in HBV-chronically infected patients receiving anti-HBV drugs

In most patients HBV DNA levels fall during therapy, the pattern of fall can vary…….



Dahari H. et al., Hepatology. 2009

The rate of viral load decline could be affected by several mechanisms

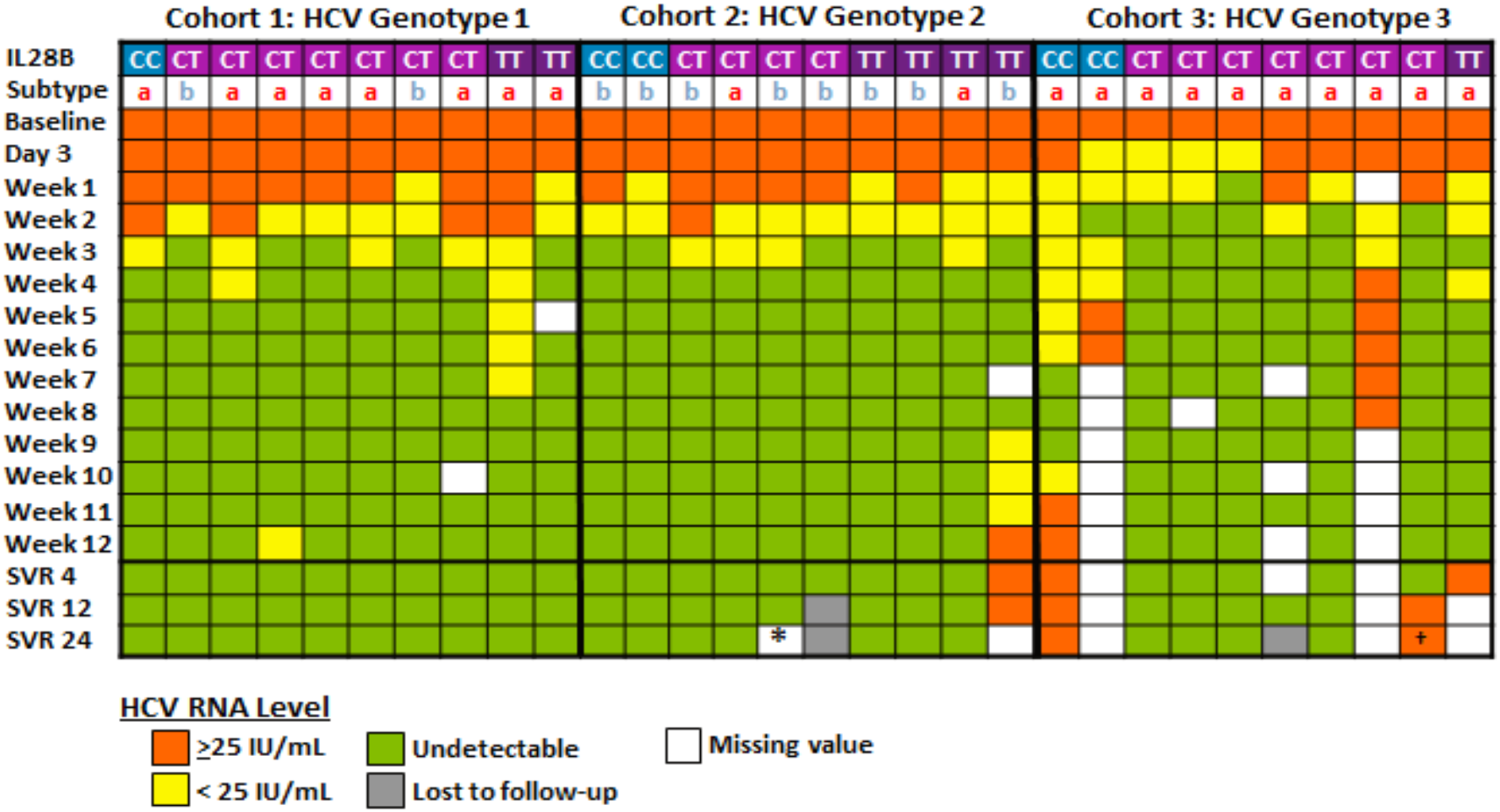


Viral eradication is considered as achieved once the predicted total HCV RNA is lower than one copy in the entire extracellular fluid volume, assumed to be 15L, which corresponds to a viral concentration of 6.7 x 10-5 HCV RNA/mL. Once the viral load is predicted to cross this boundary, HCV is considered eradicated. The cure boundary can be based on the last infected cell instead of last virion. The cure boundary makes the assumption that the HCV RNA decline rate is the same before and after it crosses the assay limit of detection (orange dotted line). This may not be correct, for instance, when resistance or reservoirs lead to a slowing of the viral decline (red dotted line), or, on the other hand, if there are mechanisms leading to an acceleration of the viral decline (due, for instance, to restored immune capabilities).

Nguyen THT and Guedj J, CTP Pharmacometrics Syst. Pharmacol. 2015

Figure 2a.

Arm 1: Ombitasvir + ABT-450/r + RBV



*Patient was lost to follow-up at PTW24 and PTW36 and returned with HCV RNA <25 IU/mL at PTW48. This patient is regarded as having reached SVR_{24} after imputation.

†Phylogenetic analysis of viral sequence indicates likely new infection with HCV subgenotype 2b

Lawitz, J Inf 2014