Supplementary Online Content

Osinusi A, Meissner EG, Lee Y-J, et al. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA*. doi:10.1001/JAMA.2013.109309

This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1: Subjects With HCV RNA Below The Level Of Quantification (part 1)

Treatment Week	Sofosbuvir + Weight-based RBV n=10 (ITT analysis)	Sofosbuvir + Weight-based RBV n=9 (per protocol analysis)
24 week treatment period – N % (95% CI)		
4	8 80 (44 -97)	8 89 (52-100)
8	9 90 (55 - 100)	9 100 (66-100)
12	9 90 (55 - 100)	9 100 (66-100)
24	9 90 (55 - 100)	9 100 (66-100)
post treatment period – N % (95% CI)		
2	9 90 (55 - 100)	9 100 (66-100)
4	9 90 (55 - 100)	9 100 (66-100)
8	9 90 (55 - 100)	9 100 (66-100)
12	9 90 (55-100)	9 100 (66-100)
24	9 90 (55-100)	9 100 (66-100)

Treatment response in part 1 subjects: ITT analysis and per protocol analysis (subjects who completed at least 8 weeks of treatment which excludes 1 patient who dropped out at week 3). Assay used was Roche assay with lower limit of quantification of 43 IU/ml. Weight based ribavirin: 1000-1200mg/day;
Abbreviations: ETR: End of treatment response, SVR: Sustained virologic response.

e Table 2: Adverse Events During the Treatment Period (Part 1)

Adverse event – N (%)	Sofosbuvir + Weight-based RBV (Part 1) n=10
Death	0
Any serious adverse event	0
Any grade 3/4 event	1 (10)
Any discontinuation owing to an adverse event	0
Ribavirin dose reduction	2 (20)
Headache	2 (20)
Anemia	4 (40)
Fatigue	4 (40)
Nausea	1 (10)
Dyspnea	1 (10)
Vomiting	0
Dizziness	0
Pruritic rash	0
Myalgia	0
Laboratory abnormality	
-Anemia	
Grade 1 (10.0-10.9 g/dL)	1 (10)
Grade 2 (9.0-9.9 g/dL)	3 (30)
Grade 3 (7.0-8.9 g/dL)	0
-Hyperbilirubinemia	
Grade 1 (1.1-1.5 x ULN)	1 (10)
Grade 2 (1.6-2.5 x ULN)	1 (10)
Grade 3 (2.5- 5 x ULN)	0
-Hypophosphatemia	
Grade 2 (2.0-2.4 mg/dL)	1 (10)
Grade 3 (1.0-1.9 mg/dL)	1 (10)
-Neutropenia	
Grade 3 (500-749/mm3)	0
Hypocalcemia (<2.04 mmol/L)	1 (10)
Hypomagnesemia (<0.63 mml/L)	2 (20)
Elevated serum creatinine (>1.1x ULN)	0

All adverse events that occurred during the study in at least 2 subjects in any group are included regardless of relatedness to study drug. Weight based ribavirin: 1000-1200mg/day,: Low dose ribavirin: 600mg/day.

e Table 3: Rates of Sustained Virologic Response Among Patients by Baseline Characteristics (Part 2)

Subgroup - no with SVR_{12} / total no (%)	Sofosbuvir + weight-based RBV (part 2) n=25	Sofosbuvir + low-dose RBV (part 2) n=25	***P values***
Race			
Black	13/18 (72)	10/23 (43)	*0.11*
Non black	4/7 (57)	2/2 (100)	*0.50*
Gender			
Male	11/18 (61)	4/14 (29)	*0.09*
Female	5/6 (83)	8/11(73)	*1.00*
Age			
≥50 years	14/21 (67)	7/17 (41)	*0.19*
<50 years	3/4 (75)	5/8 (63)	*1.00*
BMI			
>30	9/12 (75)	9/14 (64)	*0.68*
<30	8/13 (62)	3/11(27)	*0.12*
IL28B genotype			
CC	2/4 (50)	2/4 (50)	*1.00*
CT/TT	15/21 (71)	10/21 (48)	*0.21*
Knodell HAI Fibrosis			
Early Stage (0-1)	14/19 (74)	10/18 (56)	*0.31*
Advanced (3-4)	3/6 (50)	2/7 (29)	*0.59*
HCV genotype			
Genotype 1a	15/20 (75)	8/16 (50)	*0.17*
Genotype 1b	2/5 (40)	4/9 (44)	*1.00*
HCV RNA			
>800,000 IU/mL	10/16 (63)	3/14 (21)	***0.03 ****
<800,000 IU/mL	7/9 (78)	9/11 (82)	*1.00*

Rates of Sustained virologic response. by baseline characteristics in all randomized patients (ITT). There was a significantly higher rate of SVR in patients receiving weight-based RBV with baseline HCV RNA > 800,000 compared to those receiving low dose RBV. Weight based ribavirin: 1000-1200mg/day, Low dose ribavirin: 600mg/day, Abbreviations: BMI: Body mass index, HCV: hepatitis C virus, RBV: ribavirin

eTable 4: VK/PK/PD Modeling of Sofosbuvir and Ribavirin (Part 2)

A. Fitted VK modeling of 50 subjects

Parameters		**Effectiveness % (ε)**		**Loss rate of infected cells (δ)**	
	N	Median (Min, Max)	p-value	Median (Min, Max)	p-value
LDR	10	99.97 (99.69, 100)		0.34 (0.15, 0.48)	
WBR	10	99.97 (60.78, 100)	0.72	0.29 (0.008, 0.48)	0.77
REL	8	99.97 (99.51, 100)		0.34 (0.15, 0.48)	
SVR	11	99.97 (60.78, 100)	0.86	0.28 (0.008, 0.48)	0.54
GT-1a	17	99.97 (60.78, 100)		0.32 (0.008, 0.48)	
GT-1b	3	99.96 (99.69, 100)	0.51	0.32 (0.07, 0.48)	0.60
IL28B CC	5	99.98 (99.94, 99.99)		0.36 (0.07, 0.48)	
IL28B CT/TT	15	99.97 (60.78, 100)	0.21	0.28 (0.008, 0.48)	0.35
Fibrosis 0-1	13	99.97 (60.78, 100)		0.28 (0.008, 0.48)	
Fibrosis 3-4	7	99.96 (99.51, 100)	0.86	0.36 (0.12, 0.48)	0.32
Male	12	99.98 (60.78, 100)		0.34 (0.008, 0.48)	
Female	8	99.97 (99.69, 100)	0.37	0.30 (0.07, 0.48)	0.33
BMI<30	10	99.95 (60.78, 100)		0.32 (0.008, 0.48)	
BMI>30	10	99.98 (99.51, 100)	0.09	0.33 (0.15, 0.48)	0.38

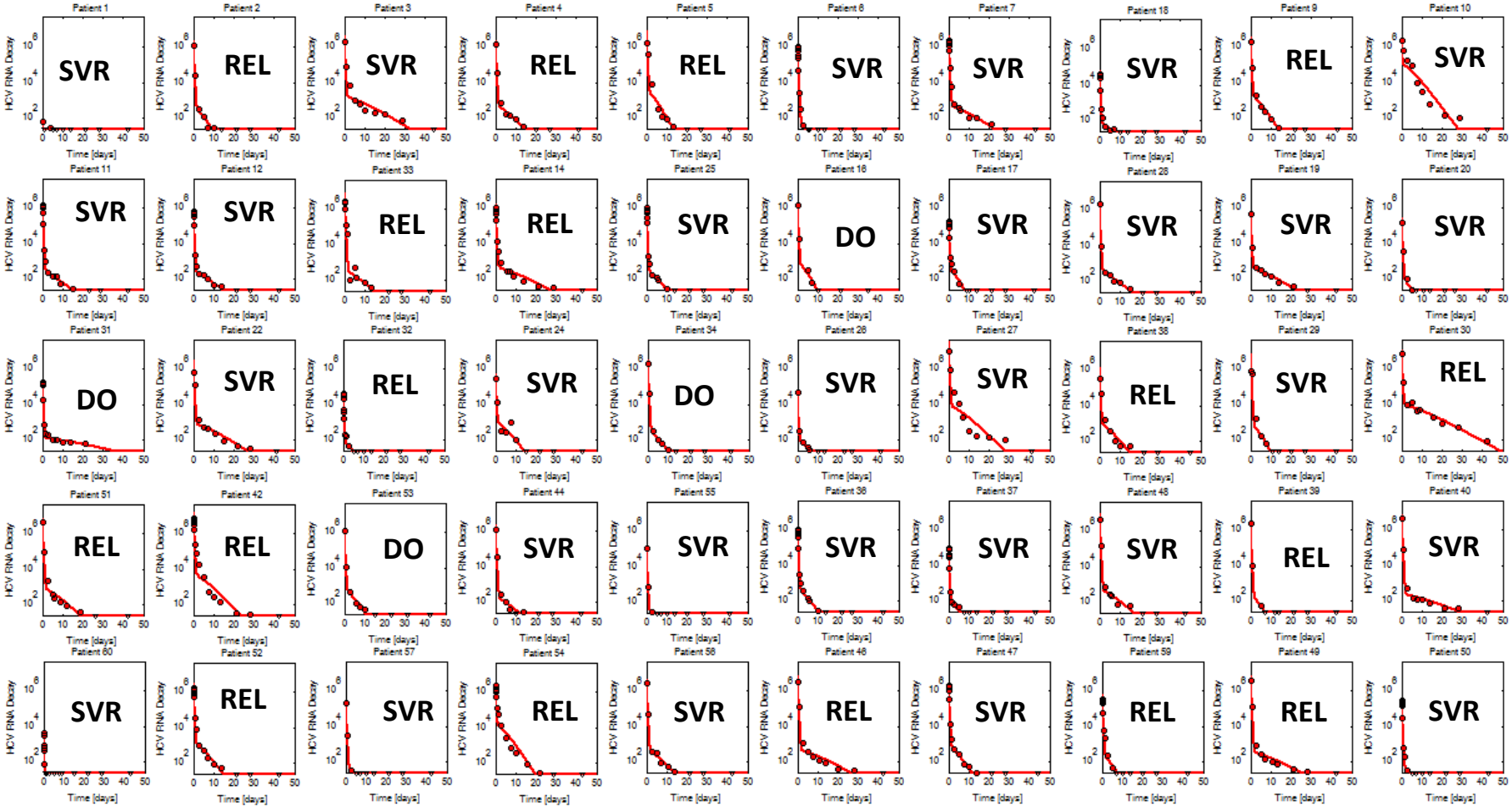
B: Fitted PK-VK model of 20 subjects

Parameters		Effectiveness % (ε)		Loss rate of infected cells (δ)		Loss rate of infected virus*(c)*	
	N	(Median (Min, Max)	p-value	Median (Min, Max)	p-value	(Median (Min, Max)	p-value
LDR	10	91.86 (78.83, 99.96)		0.21 (0.03, 0.41)		4.45 (2.26, 7.04)	
WBR	10	93.01 (68.18, 98.37)	0.53	0.20 (0.02, 0.44)	0.97	5.59 (1.88, 7.15)	0.39
REL	8	89.55 (68.18, 98.54)		0.23 (0.13, 0.39)		3.58 (1.88, 7.15)	
SVR	11	94.07 (71.34, 99.96)	0.26	0.20 (0.02, 0.44)	0.77	5.61 (4.13, 7.04)	0.009 8*
GT-1a	17	92.4 (68.18, 99.96)		0.27 (0.02, 0.44)		5.57 (1.88, 7.15)	
GT-1b	3	88.69 (78.83, 98.54)	0.85	0.15 (0.13, 0.17)	0.38	4.14 (3.58, 4.70)	0.32
IL28B CC	5	97.3 (88.41, 98.37)		0.23 (0.02, 0.41)		5.78 (3.57, 6.15)	
IL28B CT/TT	15	91.5 (68.18, 99.96)	0.20	0.21(0.02, 0.44)	1.00	5.18 (1.88, 7.15)	0.35
Fibrosis 0-1	13	93.43 (71.34, 99.96)		0.16 (0.02, 0.44)		5.56 (2.26, 7.04)	
Fibrosis 3-4	7	84.8 (68.18, 98.54)	0.28	0.23 0.17, 0.39)	0.16	3.85 (1.88, 7.15)	0.44

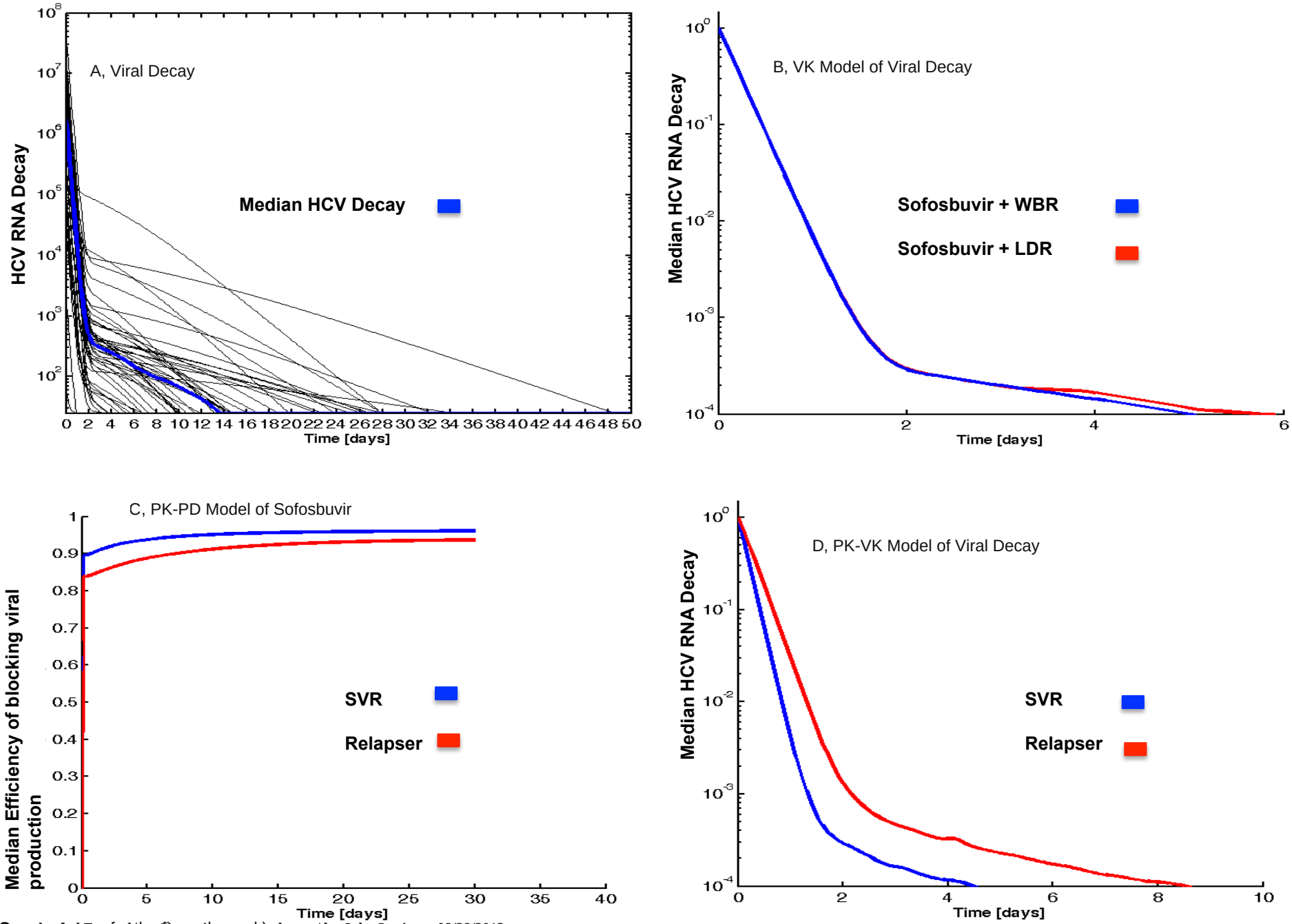
Male	12	96.86 (68.18, 99.96)		0.20 (0.02, 0.44)		5.48 (1.88, 7.15)	
Female	8	90.53 (78.83, 94.71)	0.24	0.21 (0.04, 0.33)	0.97	5.38 (2.26, 6.15)	0.91
BMI<30	10	89.95 (68.18, 98.54)		0.21 (0.04, 0.44)		5.57 (2.26, 7.15)	
BMI>30	10	95.61 (69.39, 99.96)	0.19	0.20 (0.02, 0.41)	0.68	4.94 (1.88, 7.04)	0.68

The compartments considered are: V_I *: Infectious Virus;* V_{NI} *: Non Infectious Virus;* $V = V_I + V_{NI}$ *: Viral Load;* I *: Infected Cells and* T *: Target Cells. Parameters are ε: Effectiveness of drug;* ρ *: Effect of RBV[26];* p *: Proliferation of infected cells, [27];c: Loss rate of free virus;β: De-novo infection rate;Y: Regeneration of target cells[28] and δ: Loss rate of infected cells*
A; Fitted VK modeling of all 50 randomized patients. B: VK-PK model of randomized patients (n=20)
WBR: Weight based ribavirin: 1000-1200mg/day,: LDR Low dose ribavirin: 600mg/day, GT: Genotype; REL: relapse; SVR: sustained virologic responder; BMI :body mass index

eFigure 1. Fitted HCV Decay of Individual Subjects (Part 2 of the Study)



eFigure 2. Viral Kinetic Pharmaconkintetic and Pharmacodynamics Curves



(See next page for the figure legend.)

eFigure 2. Viral Kinetic Pharmaconkintetic and Pharmacodynamics Curves (Legend)

Fitted curves for hepatitis C viral kinetics (VK) in 50 randomized participants (a and b), pharmacokinetics and pharmacodynamics (PK-PD) in 20 randomized participants (c), and pharmacodynamics and viral kinetic (PD-VK) in 20 randomized participants (d).
(a) The median fitted curves are plotted for viral decay (VK) with the overall median plotted in blue.
(b) The median viral decay curves were rapid and independent of ribavirin dosing
(c) The median value of mean efficiency of drug blocking viral production in the PK-PD model was similar in SVR vs. relapsers (p=0.26)
(d) The PK-VK model for viral decay showed a significantly higher loss rate of infectious virus in patients who achieved SVR compared to relapsers , (p=0.009)
Abbreviations: SVR: Sustained virologic responders; pharmacokinetics (PK); pharmacodynamics (PD); viral kinetics (VK).
Details of the VK/PK/PD modeling are as below:

Pharmacokinetics (PK) : Used Bateman function using parameters k_1 and k_2 for drug absorption and elimination.

Pharmacodynamics (PD): Used an intermediate compartment Z defined by $\dot{Z}(t) = aC(t) - aZ(t)$
through the Hill function $\varepsilon(t) = \frac{Z(t)^h}{Z(t)^h + IC_{50}^h}$
Viral kinetic (VK): based on the general model for HCV viral kinetics

$$\dot{V}(t) = (1-\varepsilon)(1-\rho)pI(t) - cV(t)$$

$$\dot{V}_{NI}(t) = (1-\varepsilon)\rho pI(t) - cV_{NI}(t)$$

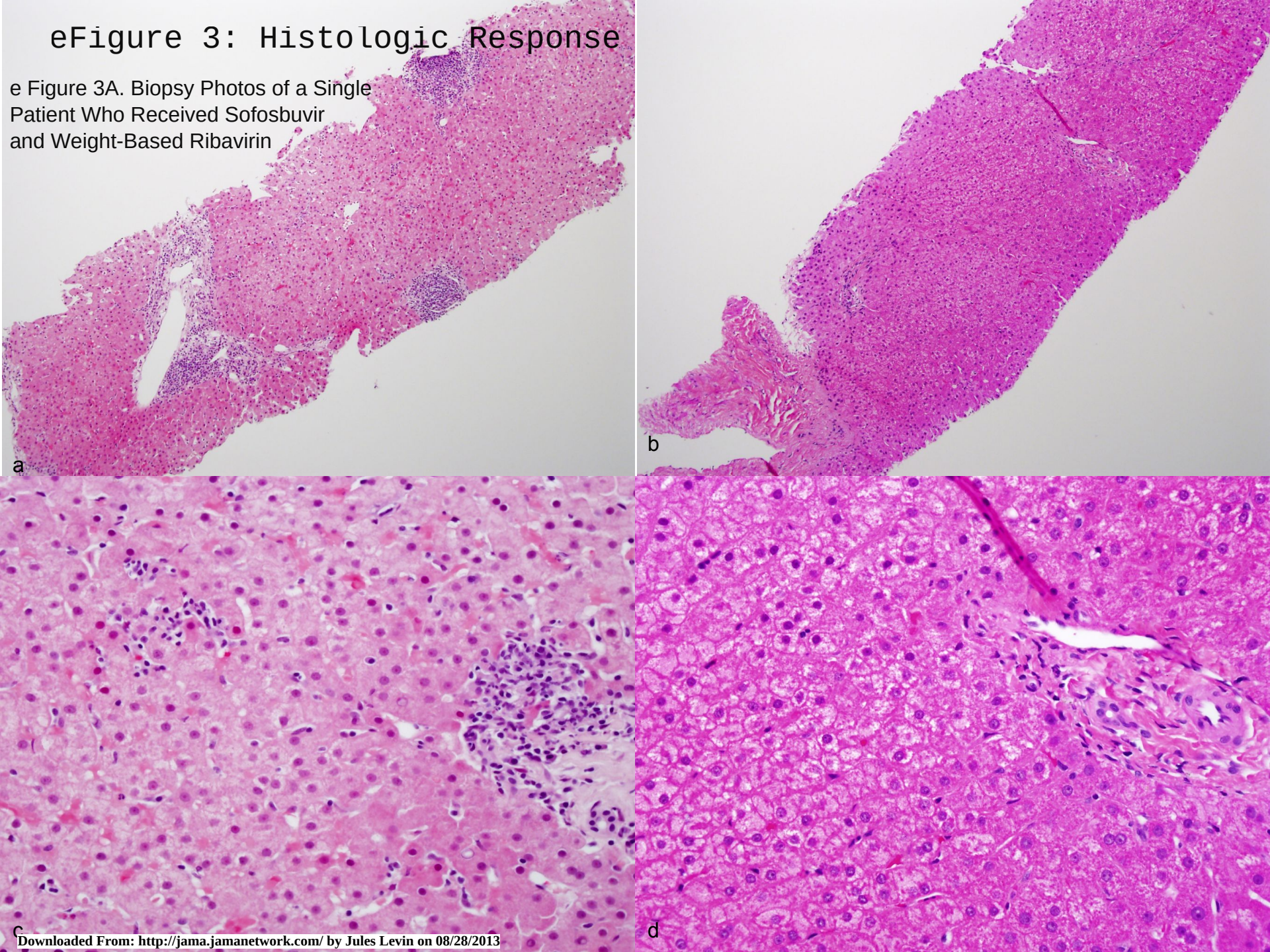
$$\dot{I}(t) = \beta T(t)V(t) + pI(t)(1 - \frac{T(t)+I(t)}{T(0)+I(0)}) - \delta I(t)$$

$$\dot{T}(t) = \gamma(1 - \frac{T(t)+I(t)}{T(0)+I(0)})$$

Data was fitted by maximum likelihood method accounting for data below the quantitation limits.For the VK model without using a PK-PD approach, only a constant drug effectiveness, infected cell loss rate and baseline viral load were fitted. Loss rate of free virus c was fixed to 5/day, regeneration rate γ to 3/day, proliferation p to 3/day, and ribavirin effect ρ was 60%. Further parameters were obtained from steady state conditions before treatment started. For a full PK-PD approach, the same parameter assumptions were used; additionally, the rate a for the intermediate compartment was log (2)/2/day and the hill parameter was 1.

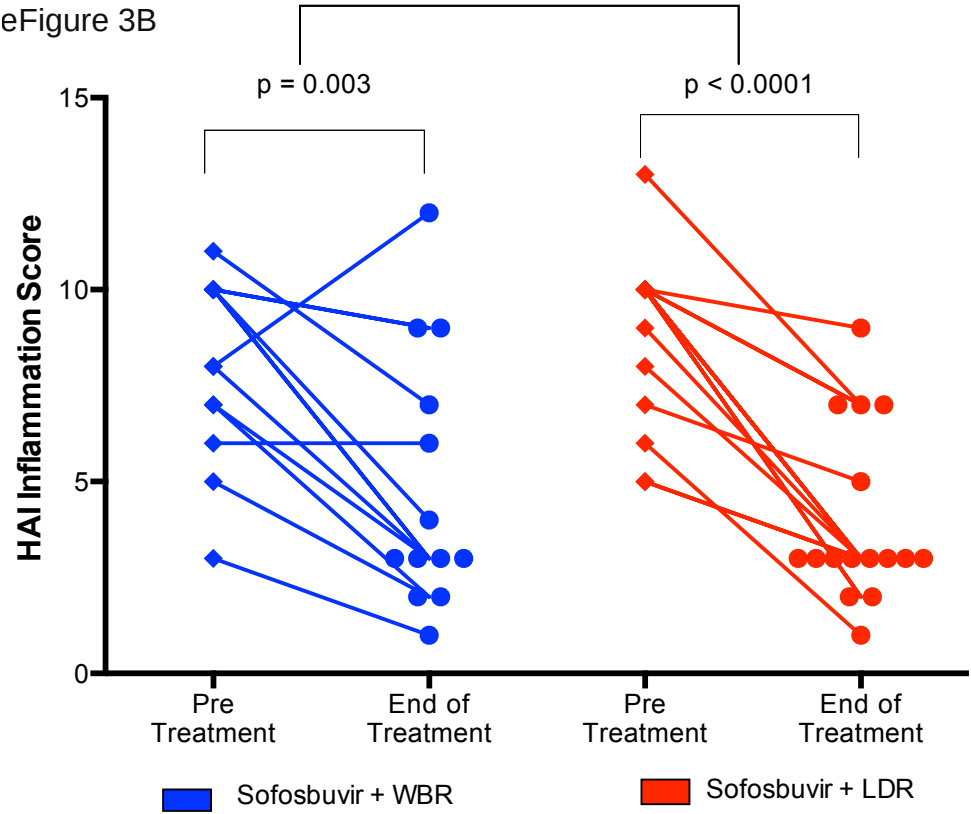
eFigure 3: Histologic Response

e Figure 3A. Biopsy Photos of a Single Patient Who Received Sofosbuvir and Weight-Based Ribavirin



eFigure 3: Histologic Response p = 0.07

eFigure 3B



efigure 3. Histologic Response (legend)

A: Biopsy photos of a single patient who received Sofosbuvir and weight-based RBV

a, c: Pre-treatment – There is moderate hepatitis, with dense portal inflammation, focal perivenular inflammation and multiple foci of spotty lobular inflammation (a: 100x; b: 400x)

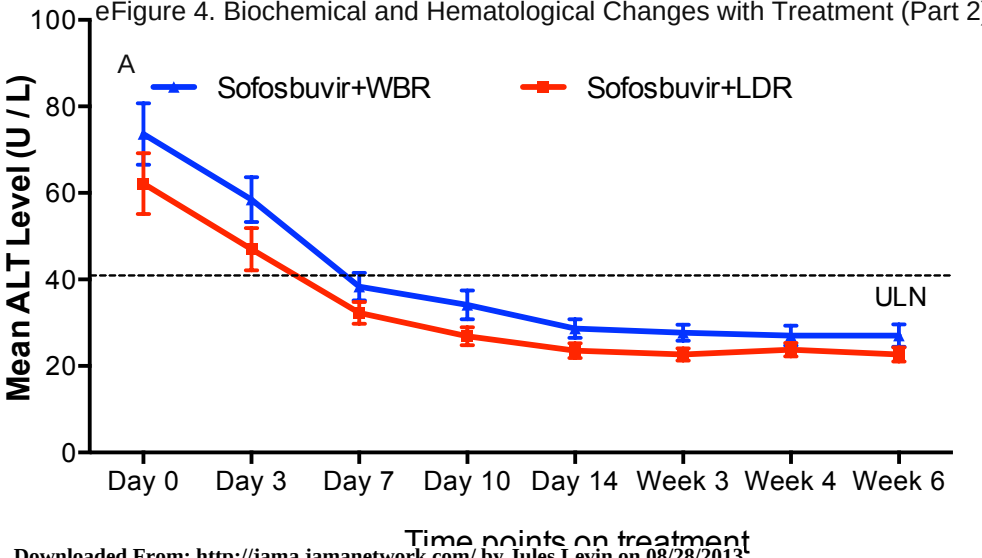
b,d: Post-treatment- There is only mild portal inflammation without interface hepatitis. The foci of lobular inflammation were very rare. (c:100x; d:400x)

B: Change in Hepatic Inflammation at the end of treatment.

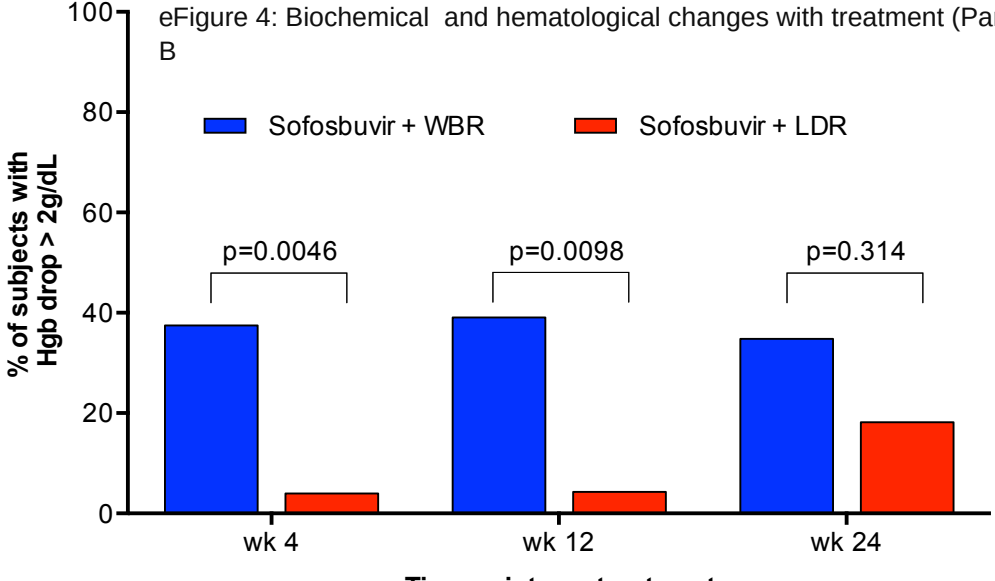
Twenty-nine participants had paired liver biopsies at the end of treatment.There was a significant improvement in the fibrosis scores at the end of treatment in all groups.This was not significantly different across groups.

WBR: Weight based ribavirin: 1000-1200mg/day;LDR Low dose ribavirin: 600mg/day,

eFigure 4. Biochemical and Hematological Changes with Treatment (Part 2)



eFigure 4: Biochemical and hematological changes with treatment (Part 2)
B



eFigure 4. Biochemical and Hematological Changes with Treatment (Part 2), legend

ALT: Alaninine aminotransferase, ULN: Upper limit of normal 41U/L
B: Hemoglobin decline over time.
Percentage of participants who experienced a hemoglobin drop of >2g/dL by a certain timepoint. There was significantly higher proportion of participants on weight based RBVwho experienced Hgb drop >2g/dl at week 4 and week 12 compared to low dose RBV. Hgb: hemoglobin.
WBR: Weight based ribavirin: 1000-1200mg/day; LDR Low dose ribavirin: 600mg/day,