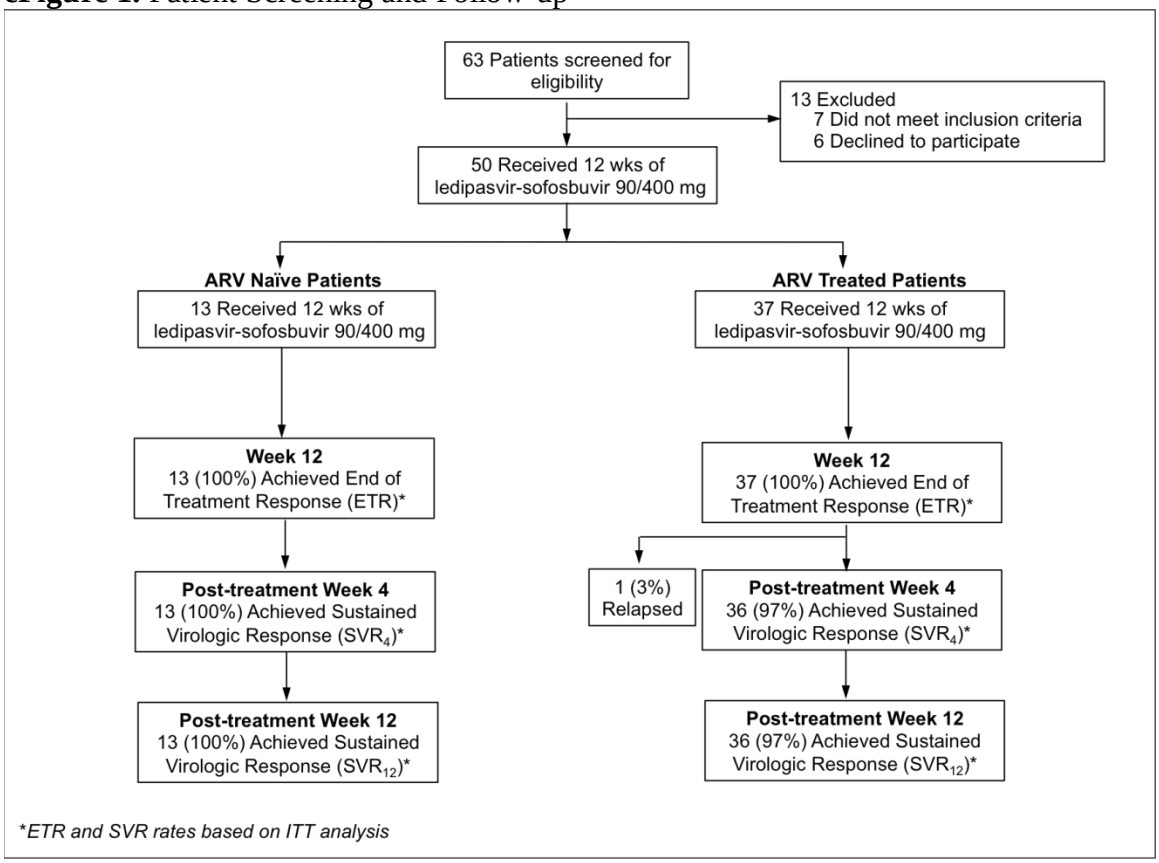
Supplementary Online Content

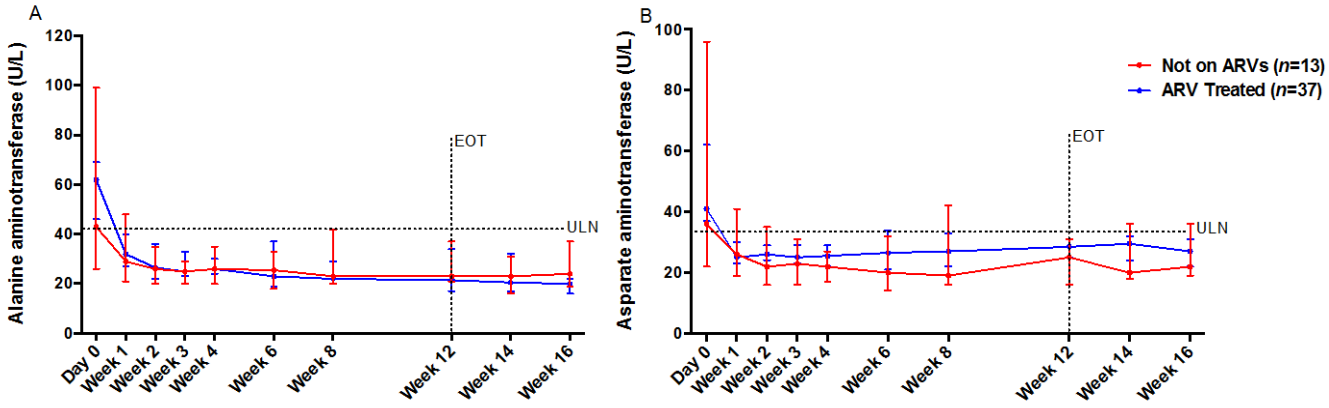
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Patient Screening and Follow-up



Screening and enrollment of the study. Sixty-three patients were screened for eligibility and fifty patients enrolled on study and received 12 weeks of a fixed dose combination of ledipasvir and sofosbuvir. No patients discontinued the study after starting study drugs. Relapse is determined at any time after end of treatment response but prior to sustained virologic response at 12 weeks post-treatment.

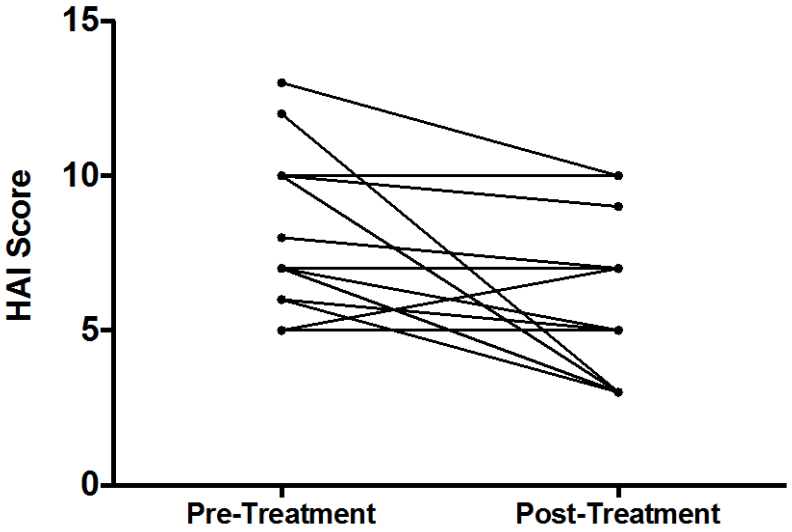
eFigure 2. Decline and Normalization in ALT and AST on Treatment



ULN: Upper Limit of Normal. ALT ULN: <41 U/L. AST ULN: <34 U/L.
EOT: End of Treatment
Median with 95% Confidence Intervals reported.
Not on ARVs n=13 at all time points except Week 3 and 6 where n=12.
ARV Treated n=37 at all time points except Weeks 2, 4, 6, 12, 16 where n=36 and Week 14 where n=34.

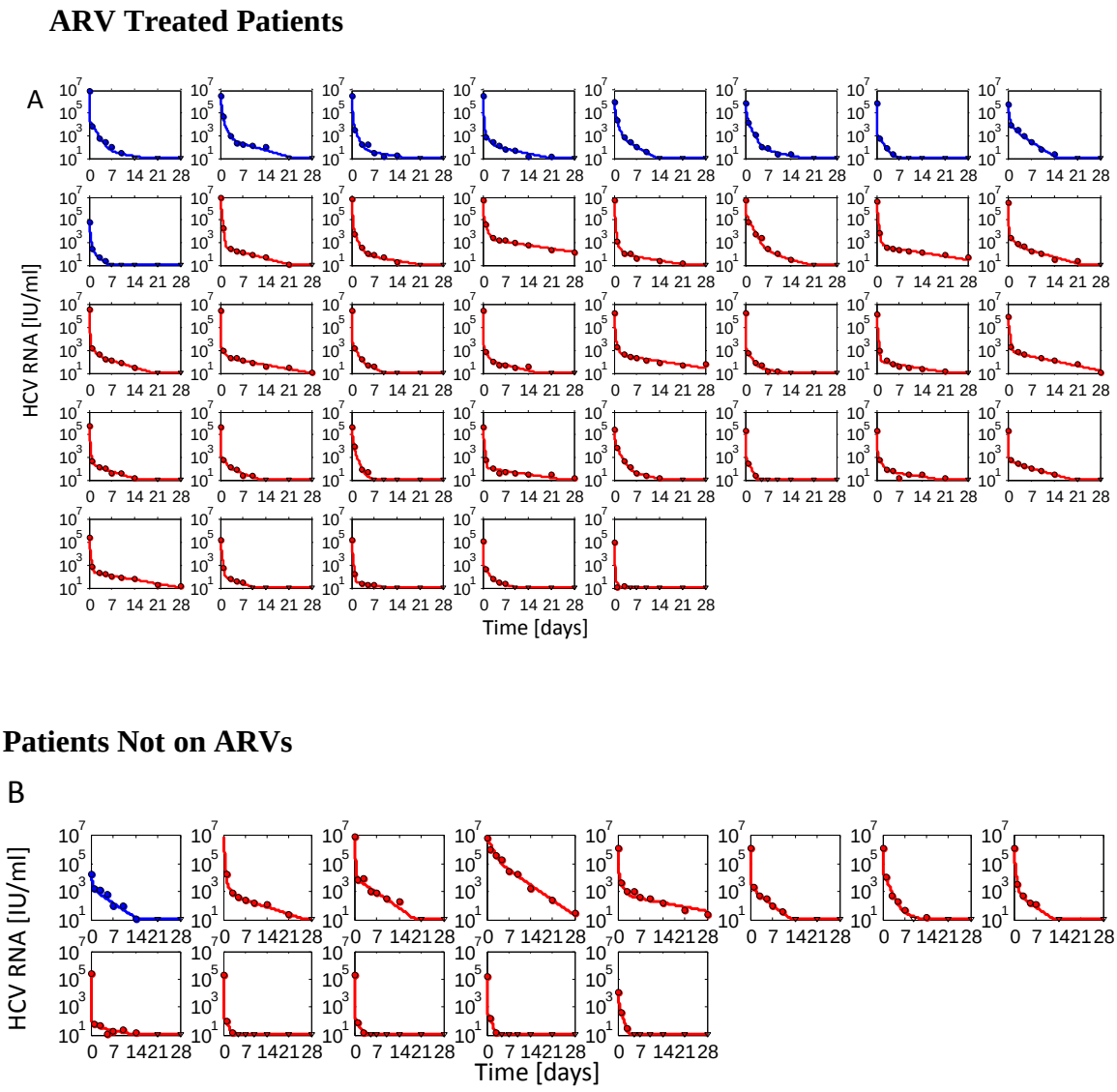
Alanine aminotransferase (ALT) levels declined to normal (Upper Limit of Normal < 40 U/L) by day 14 in 85% of patients not on antiretroviral treatment and 76% of patients on antiretroviral therapy (p=0.70). A similar pattern was observed with aspartate aminotransferase (AST) levels where levels declined to normal by day 14 in 77% of ARV naïve patients and 86% of patients on antiretroviral therapy (p=0.41).

eFigure 3. Knodell-HAI Scores From Liver Biopsies Pre- and Post-Treatment



Paired liver biopsies, pre- and post-treatment, were obtained from fourteen patients. Histology Activity Index (HAI) Knodell scores significantly decreased by a mean of 2.2 (8.07 to 5.86) (p=0.01). Knodell HAI inflammation score ranges from 0-18. A higher score indicates more inflammation.

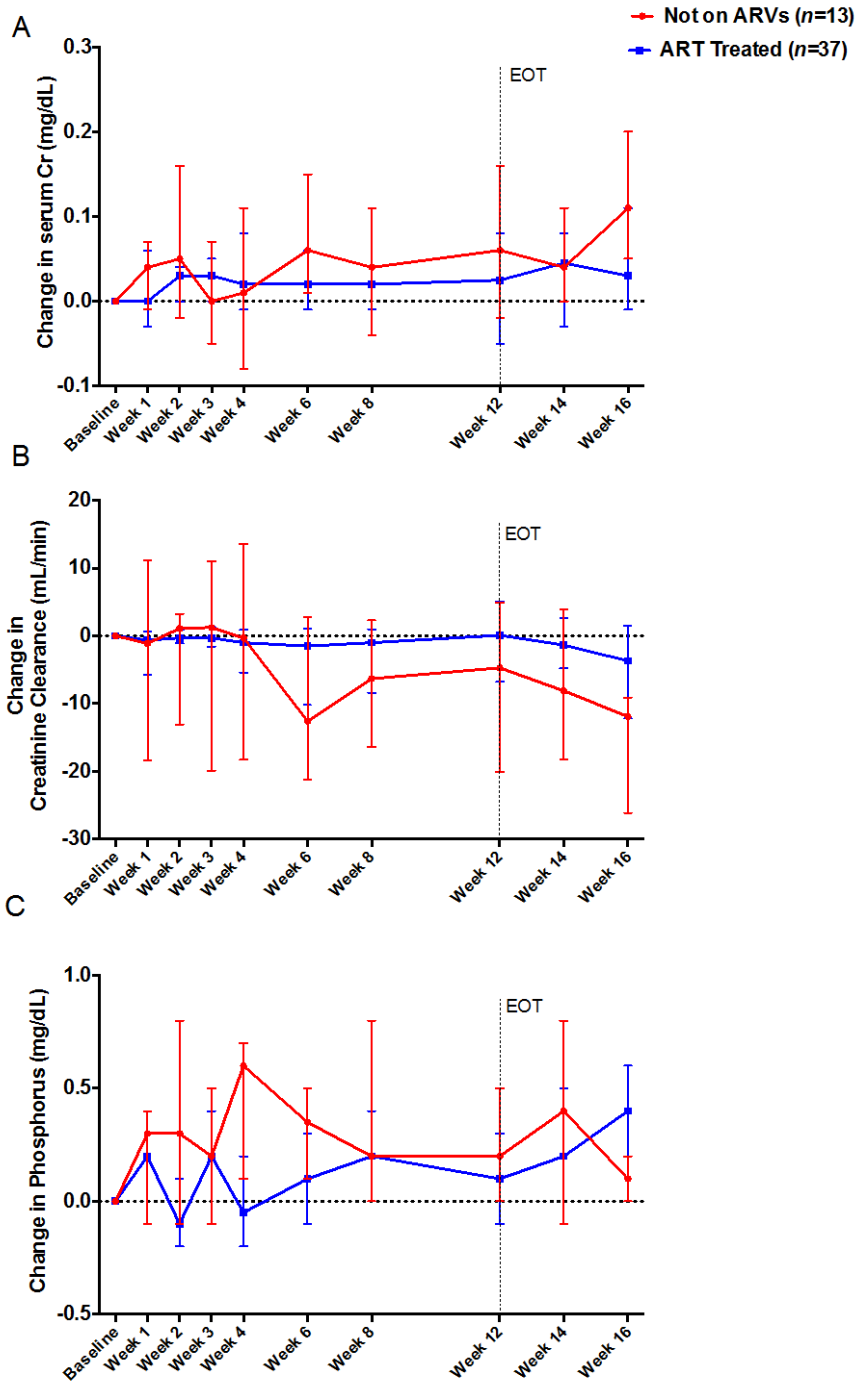
eFigure 4. Individual Viral Kinetic Modeling in Patients Treated With 12 Weeks of Ledipasvir-Sofosbuvir



Abbreviation: ARV, Antiretroviral Therapy. (A) ARV Treated Patients. (B) ARV Naïve Patients. Blue indicates a baseline CD4 T-cell count of <350 cells/mm^3. Red indicates a baseline CD4 count >350 cells/mm^3. Within each CD4 T-cell group (high or low), patients are ordered from high to low baseline HCV RNA IU/mL by Abbott. Lower limit of quantification is 12 IU/mL. Relapse patient indicated by dashed line.

Blue indicates a baseline CD4 T-cell count of <350 cells/mm^3. Red indicates a baseline CD4 count >350 cells/mm^3. Within each CD4 T-cell group (high or low), patients are ordered from high to low baseline HCV RNA IU/mL by Abbott. Lower limit of quantification is 12 IU/mL.

eFigure 5. Changes in Creatinine, Creatinine Clearance, and Phosphorus Over the Course of Treatment



Abbreviation: ARV, Antiretroviral Therapy; EOT, End of Treatment. Median change from baseline with 95% Confidence Intervals reported. (A):Not on ARVs n=13 at all time points except at Week 3 where n=12. ARV Treated n=37 at all time points except Weeks 1, 4, 16 where n=36, Week 6 n=35, Week 14 n=34 and Week 12 n=28. (B):Not

on ARVS n=13 at all time points except Week 2, 8 where n=12, Week 12 n=10 and Week 14 n=9. ARV Treated n=37 except at Weeks 1, 6, 16 n=36, Week 12 n=27 and Week 14 n=26. Not on ARVS n=13 except at Weeks 3, 6 n=12. ARV Treated n=37 except Weeks 4, 16 n=36, Weeks 1, 6, 12 n=35, Week 14 n=34.

eTable 1. Reasons for Screening Failure

Patient ID	**Reason for Screen Failure**
1	HIV Viremia: 132 copies/mL (ARV Treated Patient)
2	Decreased Albumin: 2.9 g/dL,
3	Abnormal EKF: Left bundle branch block
4	Incorrect Genotype: Genotype 2a
5	Needed shoulder surgery
6	High Hgb A1C: 10.2
7	Decreased Albumin: 2.9 g/dL

eTable 2. Median Log_{10} Decline From Baseline in HCV RNA (IU/mL) Level During Early Treatment

Treatment Time point	Not on ARVs (*n* = 13)	ARV Treated (*n* = 37)
Day 3	3.4 (2.6,4.2)	3.7 (3.5,4.0)
Day 5	3.9 (3.0,4.2)	3.9 (3.8,4.3)
Day 7	4.1 (3.0,4.2)	4.3 (4.0,4.5)
Day 10	4.2 (3.0,4.6)	4.4 (4.1,4.7)
Week 2	4.2 (3.6,5.0)	4.7 (4.3,4.8)
Week 3	4.4 (4.2,5.1)	4.8 (4.4,5.1)
Week 4	4.8 (4.2,5.4)	4.8 (4.6,5.3)

Abbreviation: ARV, Antiretroviral Therapy. Median change from Baseline in HCV RNA (IU/mL) during early treatment. Median with 95% Confidence Intervals reported.

HCV RNA declined rapidly during treatment. HCV RNA decline (log_{10}) in first 4 weeks is shown in this table. Viral decline was similar in subjects on or off of antiretrovirals.

eTable 3. HIV Viral Load >40 Copies/mL in Patients on ARV Therapy During Study

Patient ID	HIV VL >40 copies/mL	Time points	ARV Regimen	Resolution	Prior Incidents
Patient 1	594	WK 4	RPV/TDF/FTC + RALT	Yes	Yes
Patient 2	41	WK 2	EFV/TDF/FTC		
Patient 3	81	Day 1	EFV/TDF/FTC	No	Yes
	203	Day 5			
	226	WK 1			
	146	WK 2			
	103	WK 8			
	46	WK 12			
	71	WK 24			
Patient 4	44	Day 3	RPV/TDF/FTC	Yes	Yes
	68	Day 5			
	48	WK 12			
Patient 5	42	WK 8	EFV/TDF/FTC	Yes	Yes
Patient 6	64	WK 8	EFV/TDF/FTC +RALT	No	Yes
	106	WK 12			
Patient 7	91	Day 1	EFV/TDF/FTC	Yes	Yes
	44	Day 3			
	43	Day 5			
	118	WK 4			
Patient 8	60	Day 1	EFV/TDF/FTC	Yes	Yes
	159	Day 3			
	137	Day 5			
	42	WK 1			
	44	WK 2			
	54	WK 4			

Abbreviation: ARV, Antiretroviral Therapy. Resolution: HIV RNA VL became suppressed (<40 copies/mL) while on therapy. Prior Incidents: Indicates whether patient has previously experienced an increase in HIV RNA VL >40 copies/mL prior to initiation of HCV treatment.

All ARV treated patients had HIV RNA VL <40 copies/mL at baseline and at all other timepoints.

eTable 4. Changes in HIV-1 RNA Over Time in Patients Not on ARVs Who Had HIV RNA > 50 Copies/mL at Baseline

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Patient Characteristics							
HCV Genotype	1A	1A	1A	1B	1A	1A	1A
Sex	Male	Male	Male	Male	Female	Male	Female
Fibrosis Score	0	0	1	3	1	1	3
IL28B Genotype	CT	TT	TT	CC	CT	CT	TT
HIV RNA by Visit, copies/mL							
Baseline	84	65	2203	94	72	107	10069
Day 1	84	<40	2016	86	167	<40	13916
Day 3	88	109	2441	118	242	44	23544
Day 5	-	-	2525	83	173	41	16032
Day 7	<40	<40	-	153	73	<40	11917
Week 2	132	150	2030	246	83	<40	4108
Week 4	42	55	2642	138	77	<40	761
Week 8	193	<40	2009	<40	78	<40	833
Week 12	<40	<40	1262	60	213	40	833
Week 24	52	53	2198	134	<40	40	1951

Abbreviation: ARV, Antiretroviral Therapy

eTable 5. CD4 T-cell Counts and CD4 T-Cell Percent Over Time

		Not on ARVs (*n* = 13)	ARV Treated (*n* = 37)	All Patients (*n* = 50)
CD4 count (cells/mm^3)				
	Baseline	687 (525-944)	576 (372-740)	592 (440-789)
	Week 1	795 (520-911)	564 (431-778)	623 (433-884)
	Week 2	715 (530-911)	571 (429-785)	641 (490-841)
	Week 4	821 (523-947)	490 (413-701)	549 (424-857)
	Week 8	843 (524-1027)	602 (405-736)	630 (434-882)
	Week 12	731 (577-971)	552 (427-759)	626 (431-857)
	Week 24	729 (498-959)	593 (389-869)	602 (458-897)
CD4%				
	Baseline	35 (28-51)	31 (24-40)	32 (25-41)
	Week 1	34 (28-54)	30 (23-36)	32 (25-42)
	Week 2	32 (28-53)	32 (24-40)	32 (26-42)
	Week 4	33 (26-52)	29 (24-39)	30 (25-43)
	Week 8	35 (29-54)	29 (23-38)	31 (24-42)
	Week 12	36 (29-52)	31 (24-39)	31 (25-44)
	Week 24	32 (25-52)	31 (23-36)	31 (23-40)

Abbreviation: ARV, Antiretroviral Therapy.
Median with IQR reported.

eAppendix 1. Inclusion and Exclusion Criteria

Inclusion Criteria

1. Eighteen years of age or older at screening.

2. HCV treatment-naïve, as defined as no prior exposure to any IFN, RBV, or other approved or experimental HCV-specific direct-acting antiviral agent.

3. Participants must be willing to practice either:
    a. Abstinence from sexual intercourse or
    b. At least 2 forms of contraception including one barrier method from 2 weeks prior to Day 0 through 30 days after the last dose is received.
        i. Female partners of male study subjects may rely upon hormonal contraception as one of the 2 methods; however female study subjects may not.

4. Chronic hepatitis C infection defined as one of the following:
    a. Positive for anti-HCV antibody, HCV RNA, or an HCV genotype at least 6 months before screening, and positive for HCV RNA and anti-HCV antibody at the time of screening or

    b. Positive for anti-HCV antibody and HCV RNA at the time of screening with a liver biopsy consistent with chronic HCV infection (or a liver biopsy performed before enrollment with evidence of chronic hepatitis C disease, such as the presence of fibrosis).

5. HIV treatment status:
    a) Documented HIV infection, ARV untreated for >8 weeks preceding dosing and having either:
        1) a CD4 T-cell count $\geq$ 500 cells/mm^3 within 8 weeks of Day 0 or
        2) an HIV viral load less than 500 copies/mL with a stable CD4 count for at least 3 months.
    b) Documented HIV infection on a stable, protocol-approved, ARV regimen for $\geq$ 8 weeks prior to dosing and is expected to continue the current ARV regimen through the end of study with all of the following:
        1) a CD4 T-cell count >100 cells/mm^3
        2) a documented plasma HIV-1 RNA level less than the level of detection for at least8 weeks preceding dosing. If the lower limit of detection of the local HIV-1 RNA assay is <50 copies/mL (e.g., <20 copies/mL), the Screening plasma HIV-1 RNA level cannot exceed 50 copies/mL.
        3) HIV ARV agents including only combination regimens consisting of medications from the following list: tenofovir (TDF), emtricitabine (FTC), efavirenz, raltegravir, and rilpivirine administered according to their manufacturer's prescribing information. (reference Section 10.3 for additional information)

6. Documentation of hepatitis C genotype 1a, 1b or mixed 1a/1b.

7. Absence of cirrhosis, defined as one of the following:
    a. A liver biopsy performed within 36 calendar months of screening showing absence of cirrhosis.
    b. FibroTest® score of <0.48 AND APRI of <1 performed during the 8 weeks preceding dosing (In the absence of a definitive diagnosis of presence or absence of cirrhosis by the above criteria, a liver biopsy is required).
8. Able to effectively communicate with the Investigator and other center personnel.
9. Willing to give written informed consent and comply with the study restrictions and requirements.
10. If opioid-dependent, subjects must be participating in a supervised treatment.
11. Participants must have a primary medical provider outside of OP8 and the NIH for medical management.

Exclusion Criteria

Subjects who meet any of the following exclusion criteria are not to be enrolled in this study:

1. Current or prior history of any of the following:
    a. Clinically significant illness (other than HCV) or any other major medical disorder that may interfere with subject treatment, assessment or compliance with the protocol; subjects currently under evaluation for a potentially clinically-significant illness (other than HCV) are also excluded.
    b. Gastrointestinal disorder or post-operative condition that could interfere with the absorption of the study drug.
    c. Poor venous access interfering with required study blood collection.
    d. Clinical hepatic decompensation (i.e., ascites, encephalopathy or variceal hemorrhage).
    e. Solid organ transplantation.
    f. Significant pulmonary disease, significant cardiac disease or porphyria.
    g. Unstable psychiatric disease (Subjects with psychiatric illness that is well-controlled on a stable treatment regimen or currently not requiring medication may be included).
    h. Any malignancy or its treatment that in the opinion of the PI may cause ongoing interference with host immunity; subjects under evaluation for malignancy are not eligible.
    i. Significant drug allergy (such as anaphylaxis or hepatotoxicity).
    j. Chronic liver disease of a non-HCV etiology (e.g., hemochromatosis, Wilson's disease, alfa-1 antitrypsin deficiency, cholangitis).
    k. Patients with renal impairment or uncontrolled medical problems that could place them at high risk for developing renal impairment.

2. Positive test at screening for either HBsAg or quantifiable HBV DNA (completed only if necessary to rule out chronic HBV).

3. Current use of non-protocol approved ARVs.
4. A new AIDS-defining condition diagnosed within 30 days prior to screening or active, serious infection (other than HIV or HCV) requiring parenteral antibiotics, antivirals or antifungals within 30 days prior to Day 0.
5. Cirrhosis of the liver.
6. Screening or baseline ECG with clinically significant ECG findings.
7. Abnormal hematological and biochemical parameters, including:
    a. Neutrophil count <750 cells/mm^3
    b. Hemoglobin <9 g/dL. If Hgb < 11g/dL in women and < 12 g/dL in men other causes of anemia should be excluded as medically indicated.
    c. Platelet count ≤50,000 cells/mm^3
    d. Estimated GFR (calculated by the CKD-EPI equation) <50 mL/min/per 1.73 m^2 if not on ARV or <60 mL/min if on ARVs
    e. ALT or AST ≥10 times ULN
    f. Serum lipase ≥1.5 times ULN (at screening or during the screening period)
    g. Direct bilirubin ≥1.50 times ULN
    h. Albumin ≤3.0 g/dL
    i. INR ≥ 1.5 x ULN unless subject has known hemophilia or is stable on an anticoagulant regimen affecting INR.
8. Donation or loss of more than 400 mL blood within 8 weeks prior to first dose administration.
9. Poorly controlled diabetes mellitus, indicated by hemoglobin A1C > 10% at screening for known diabetics.
10. Known hypersensitivity to, GS-5885, GS-7977, or formulation excipients.
11. Pregnant/Breastfeeding women.
12. Co-enrollment in other clinical trials is restricted, and requires approval of the Investigator. Study staff should be notified of co-enrollment status.
13. Need for use of the following medications from 21 days prior to the start of study drugs through the end of treatment:
    a. Hematologic stimulating agents (e.g. erythropoiesis-stimulating agents (ESAs); granulocyte colony stimulating factor (GCSF); thrombopoietin (TPO) mimetics)
    b. Chronic systemic immunosuppressants including, but not limited to, corticosteroids (prednisone equivalent of > 10 mg/day for > 2 weeks), azathioprine, or monoclonal antibodies (e.g., infliximab)
    c. Investigational agents or devices for any indication
    d. Medications for disease conditions **excluded** from the protocol (e.g., active cancer, transplantation) are not listed under this Concomitant Medication section and are disallowed in the study.

e. Concomitant use of certain medications or herbal/natural supplements per PI discretion expected to result in pharmacokinetic interactions resulting in increases or decreases in exposure of study drug(s) as listed in Table 9 of this protocol.

eAppendix 2. Study Medication Stopping Criteria

Subjects will be considered treatment failures and will discontinue GS-7977/GS-5885 FDC if they meet any of the following criteria while taking study drugs:

- Serum HCV RNA greater than LLOQ after 2 prior consecutive HCV RNA values less than the LLOQ
- Greater than a 1 $\log_{10}$ increase in serum HCV RNA from nadir
- Less than a 2 $\log_{10}$ decline in HCV RNA after 4 weeks of treatment
- Serum HCV RNA ≥ LLOQ after 8 weeks of treatment

If any of these should occur in a patient who is currently on study drugs, the subject should return within one week for a confirmatory test. If the confirmatory test also meets the same criteria, the subject will be considered a treatment failure and should be discontinued from therapy. The anticipated clinical impact of discontinuation should be discussed in advance with the Sponsor Medical Monitor if possible, particularly if discontinuation is thought to pose a risk to the overall clinical wellbeing of the subject. Those who are discontinued will continue to follow the general study schedule of assessments unless unwilling to do so, in which case they may be seen at least every 12 weeks for safety and research labs until the end of the study. Subjects will be followed closely for resolution of active laboratory abnormalities or adverse events which are considered related to the study agents prior to starting the revised schedule.

Subjects who meet any of the following laboratory criteria must stop all study medication(s):

- Elevation of ALT >5x OR AST >5x Day 0, confirmed by immediate repeat testing
- Abnormal elevation of ALT >3 x Day 0 *and* total bilirubin >2 x ULN, confirmed by immediate repeat testing
- Elevation of ALT >15 x ULN confirmed by immediate repeat testing
- Any Grade 3 or greater rash associated with constitutional symptoms
- Any Grade 4 event assessed as related to treatment with GS-7977 or GS-5885

eAppendix 3. Description of Viral Kinetic Modeling

Viral kinetic (VK) modeling with a multiscale model was performed in all participants who participated in the study. Our model combines approaches from two recently published multiscale models for HCV viral kinetics (1,2). We started with an extended version of the standard model, the so called ICCI (Intracellular and Cellular Infection Model) (1). This model takes the intracellular replication cycle into account by introducing two new compartments inside the infected cells compartment. The first compartment describes the number of intracellular viral genomic units (positive genomic RNA strands) (R) that are available for transcription and translation per infected cell and the second compartment describes the number of replication units (U; negative-strand/double-stranded RNA) that are available for synthesis of genomic RNA. During untreated chronic infection, R serves as a resource for the formation of replication units with rate β while intracellular RNA strands will be produced by the replication units with rate α. During treatment, the total treatment effect is reflected by the relative reduction of the basic reproductive ratio given by $1-(1-\varepsilon_1)(1-\varepsilon_2)/(1+k)$, where ε_1 is the rate of blocking the viral production ($0 \le \varepsilon_1 \le 1$), ε_2 is the rate of blocking the synthesis of intracellular RNA ($0 \le \varepsilon_2 \le 1$) and k is a factor that enhance the loss rate of intracellular RNA mimicking an approach for multiscale modeling by Guedj et al. (2). Our viral kinetic model is described by the differential equations system:

$$V' = (1-\varepsilon_2)\rho RI - cV$$
$$I' = b(1+\eta)VT - \delta I$$
$$T' = r\left(1 - \frac{T+I}{T_0 + I_0}\right)$$
$$R' = (1-\varepsilon_1)\alpha U - (1+k)R$$
$$U' = \beta R\left(1 - \frac{U}{U_{max}}\right) - \gamma U$$

V : free virus
I : infected cells
T : target cells

ρ : export loss rate
c : loss rate of free virus
b, η : de novo infection
δ : loss rate of infected cells
r : regeneration of target cells
γ : degradation rate

Data of the first 4 weeks were fitted by maximum likelihood method accounting for data below the quantification limit and with fixed parameters $\gamma = 3/\text{day}, r = 3/\text{day}, \frac{U_{max}}{U_0} = 1.001$. By fitting the individual viral kinetic data to this model, estimators for the overall treatment effect, the infected cell loss rate and the loss rate of free virus were derived.

References

1. Guedj, J. & Neumann, A.

   Understanding hepatitis C viral dynamics with direct-acting antiviral agents due

to the interplay between intracellular replication and cellular infection dynamics *Journal of theoretical biology, Elsevier,* **2010**, *267*, 330-340

2. Guedj, J.; Dahari, H.; Rong, L.; Sansone, N. D.; Nettles, R. E.; Cotler, S. J.; Layden, T. J.; Uprichard, S. L. & Perelson, A. S.
Modeling shows that the NS5A inhibitor daclatasvir has two modes of action and yields a shorter estimate of the hepatitis C virus half-life
Proceedings of the National Academy of Sciences, National Acad Sciences, **2013**, *110*, 3991-3996