Protocol Synopsis

Study title. Direct Acting Antiviral Effects on the Liver (deLIVER Study)

NCT number: NCT02938013

IND number: 132393

Study phase: Phase 3b

Study Design: Open-label, partially-randomized plasma and liver sampling study to assess HCV kinetics during treatment with two (Sofosbuvir/Velpatasvir) or three (Sofosbuvir/Velpatasvir/Voxilaprevir) direct acting antivirals (DAAs)

Cohort 1: Paired liver biopsies on Day 0 and Day 7

Antiretroviral therapy (HIV seropositive only)													
Step 1	Step 2								Step 3			Step 4	
	SOF/VEL/VOX or SOF/VEL								SOF/VEL			Follow-up	
	Entry/D0	D1	D2	D3	D4	D5	D6	D7	WK4	WK8	WK12	WK16	WK24
Screening/ Randomization	Liver BX	VK	VK	VK	VK			Liver BX	VK	VK	VK	VK	VK
	VK							VK					

Cohort 2: Paired liver biopsies on Day 0 and Day 4

Antiretroviral therapy (HIV seropositive only)													
Step 1	Step 2								Step 3			Step 4	
	SOF/VEL/VOX or SOF/VEL								SOF/VEL			Follow-up	
	Entry/D0	D1	D2	D3	D4	D5	D6	D7	WK4	WK8	WK12	WK16	WK24
Screening/ Randomization	Liver BX	VK	VK	VK	Liver BX			VK	VK	VK	VK	VK	VK
	VK												

VK, viral kinetics; BX, biopsy; SOF, sofosbuvir, VEL, velpatasvir; VOX, voxilaprevir; D; day; WK, week.

Study duration:

Step 1	Screening/ Randomization, up to 42 days (6 weeks)
Step 2	Paired liver biopsy for intrahepatic HCV dynamics, 7 days (1 week); intensive viral kinetics (VK) plasma and liver assessment
Step 3	HCV treatment, 77 days (11 weeks); continuation treatment with two DAAs (Sofosbuvir/Velpatasvir)
Step 4	Post-treatment follow up, 84 days (12 weeks)
Total	Up to 30 weeks

Sample size: 15 participants (up to 20 screened to account for screen failures)

Population: Adults ages ≥ 18 years to ≤70 years with HCV genotype 1a infection with no cirrhosis and no prior HCV treatment (treatment naïve). Five participants with HIV infection taking protocol-specified antiretroviral therapy and ten participants without HIV infection.

Cohort assignment: Nine subjects will be assigned to Cohort 1 as follows: 3 subjects in Arm A, 3 subjects in Arm B, and 3 subjects in Arm C. The subsequent six subjects will be enrolled into Cohort 2 as follows: 2 subjects in Arm A, 2 subjects in Arm B, and 2 subjects in Arm C.

Abstract

Treatment of chronic hepatitis C virus infection with direct acting antivirals (DAAs) is highly effective in persons with and without HIV infection when administered for durations of at least 12 weeks. Shorter durations of treatment are associated with viral persistence in the liver and post-treatment relapse in some but not all patients. In general, measurement of the magnitude and rapidity of the viral kinetic response in the blood is not predictive of persistence of the virus in the liver, leading to post-treatment relapse. Little is known about the role of the intrahepatic viral kinetic during antiviral treatment or about the contribution of the patient immune system in control of the virus within the liver. In addition, the potential contribution of an additional antiviral with a different mechanism of action to intensify viral clearance of the liver has not been evaluated. The present study will assess the viral kinetic response in the plasma and the liver of 15 individuals randomized to treatment with two or three direct acting antivirals over a one week dosing period. All participants will undergo core needle liver biopsy at entry/day 0 prior to taking antivirals and on treatment day 7 (cohort 1) or treatment day 4 (cohort 2) to assess intrahepatic viral kinetics using the technique of single cell laser microdissection. To examine the role of HIV coinfection on viral kinetics, patients with HCV genotype 1a with and without HIV coinfection will be enrolled; to minimize heterogeneity, all HIV-infected patients will be required to be receiving protocol-specified antiretrovirals. The effect of HIV infection will be examined by comparison of the plasma and liver viral kinetics in 5 patients with HIV/HCV and 5 participants with HCV alone treated with the combination of two oral antivirals for 7 days. The effect of the addition of a third antiviral which inhibits HCV NS3/4A protease to the standard treatment with two antivirals which inhibit HCV NS5A and NS5B will be examined by randomizing patients with HCV monoinfection to treatment with two or three DAA over a 7 day period to assess plasma and liver viral kinetics. All participants will be offered standard of care treatment with two antivirals for an additional 11 weeks to complete a 12 week treatment course and will be followed for 12 weeks after stopping treatment to determine HCV treatment outcome.

1.0 Hypothesis

Hepatitis C virus (HCV) cure, sustained virologic response (SVR) is the consequence of the clearance of non-uniform clusters of HCV-infected cells and a decrease of intracellular HCV RNA per infected cell, which are in turn modified by the associated intrahepatic immune response in the first week of therapy in subjects with HCV with and without HIV coinfection.

2.1 One week after treatment with direct-acting antivirals, the decline in intrahepatic and intracellular HCV RNA will be greater in patients treated with an NS3 protease inhibitor in combination with inhibitors of NS5A and NS5B compared to those treated with the latter two inhibitors alone.

2.2 One week after treatment with direct-acting antivirals, the decline in intrahepatic and intracellular HCV RNA quantity will be greater in persons with HCV compared to those with HIV/HCV coinfection.

2.0 Objectives

3.1 Primary Objective: The primary objective is to estimate the 1-week change in the proportion of HCV-infected hepatocytes in participants with HCV monoinfection and HIV/HCV coinfection on therapy with two or three DAAs with different mechanisms of action using single cell laser microdissection (scLCM).

3.2 Secondary Objectives:

3.2.1. Estimate the change over the first week in plasma HCV RNA in subjects with HCV monoinfected and HIV/HCV coinfected participants on therapy with two or three DAAs

3.2.2. Estimate the 1 week change in the amount of HCV RNA per infected hepatocyte using scLCM on liver biopsy specimens, obtained just prior to treatment initiation (pre-treatment), and after the first week of DAA therapy.

3.2.3. Estimate the change in the proportion of HCV-infected hepatocytes that express interferon-stimulated genes (ISGs) within the first week of DAA therapy using scLCM.

3.2.4. Measure the change in expression of ISGs in non-parenchymal intrahepatic immune cells (Kupffer cells, plasmacytoid dendritic cells) within the first week of DAA therapy using scLCM.

3.3 Exploratory Objectives:

3.3.1. Estimate the 1 week change in expression of ISGs from peripheral blood mononucleated cells (PBMCs) within the first week of DAA+RBV therapy using scLCM.

3.3.2. Compare sequence(s) of HCV protease, nonstructural protein 5A (NS5A), and nonstructural protein 5B (NS5B) depending on the peripheral sequence) of intrahepatic HCV RNA in single cells and bulk tissue, before and during week 1 of DAA+RBV therapy.

3.3.3. Estimate the week 1 change in the sizes and numbers of HCV-infected clusters on DAA therapy to test whether clearance of HCV-infected hepatocytes occurs in spatially random patterns or within specific clusters.

3.0 Background

4.1 Disease Under Study

HIV infects approximately 30 million people worldwide and more than 1 million people in the US [1]. While antiretroviral therapy (ART) has dramatically decreased AIDS-related mortality, people living with HIV (PLWH) still have higher mortality than uninfected people. Liver-related mortality has consistently been found to be among the leading causes of death in PLWH in the US during the ART era, and HCV coinfection disproportionately contributes to liver disease in this population [2, 3]. Because of common routes of transmission, between 25-80% of PLWH in the US have been reported to be coinfected with HCV [4]. HIV worsens HCV in several ways: i) by decreasing the likelihood of spontaneous clearance; ii) by increasing the HCV RNA level, iii) by accelerating the progression of HCV-related liver disease; and iv) by worsening the response of HCV to interferon (IFN)-based treatments [4]. Improved HCV therapies have reduced the gaps in efficacy between HCV mono-infected persons and HIV/HCV co-infected persons when given for standard durations of 12 weeks; however, whereas shortened therapy has delivered promising results for HCV mono-infected persons, they have been disappointing for HIV/HCV co-infected persons.

HCV chiefly replicates in liver hepatocytes: HCV eradication is the primary clinical outcome in HCV treatment, a composite phenomenon that reflects the suppression of new hepatocyte infections and the clearance of already infected hepatocytes. There has been rapid growth in the development of DAAs that target the intracellular lifecycle of HCV to suppress viral replication, i.e., new hepatocyte infections. In combination, DAAs have few side effects and deliver high rates of SVR. However, questions remain about the biology of HCV clearance and how it is impacted by treatment duration. Since, conceptually, duration of therapy hinges upon suppressing viral replication for sufficient time to allow host clearance of HCV-infected hepatocytes, combination DAA therapy is anticipated to result in complete suppression of HCV replication that will permit accurate measurements of the rate of clearance of HCV-infected hepatocytes. In addition, host clearance of HCV-infected hepatocytes is largely presumed to be due to the immune response; therefore, studies linking host immunity with the rate of clearance of HCV-infected hepatocytes in a situation without IFN may underscore mechanisms that can be targeted to shorten therapy in the future.

A major challenge in mechanistic studies of HIV/HCV coinfection is the absence of a representative animal model. In addition, *in situ* studies of human HCV infection have been difficult to develop due to technical hurdles. We have optimized a technique that integrates a narrowly focused laser with a fully automated light microscope (laser capture microdissection; LCM) to study single hepatocytes in the HCV-infected liver. Our team used scLCM to quantify the proportion of infected hepatocytes in subjects with chronic HCV, and we found that between 21-45% of hepatocytes were HCV-infected [5]. Our data are consistent with earlier studies of *in situ* HCV infection, but with the added advantage that host mRNA can be quantified in the same cells. Pal et al. used *in situ* hybridization to identify that approximately 40% of hepatocytes had HCV RNA [6]. Similarly, Liang et al. used 2-photon excitation of Q-dot probes to detect hepatic HCV antigens and RNA, reporting that between 1.7-21.6% of hepatocytes are infected [7]. Furthermore, because scLCM permits study of host gene expression, it can be employed to quantify immune responses to HCV and metabolic responses to study drugs. scLCM, therefore, is a validated measure to study HCV infection in human liver tissue, and could feasibly be applied to the study of HCV clearance with combination DAA therapy.

4.2 Combination DAA Therapy

Sofosbuvir/Velpatasvir (SOF/VEL). Sofosbuvir is an oral, nucleotide analogue inhibitor of HCV NS5B polymerase with demonstrated, potent antiviral activity in vitro and in vivo against HCV genotype 1, 2, 3, 4, 5, and 6 (pan-genotypic). SOF was approved by the US Food and Drug Administration (FDA) in October 2013 and is widely available across the world including as a generic agent manufactured in India. Velpatasvir (VEL) is an oral, second generation inhibitor of HCV NS5A with demonstrated potent antiviral activity in vitro and in vivo against HCV genotype 1, 2, 3, 4, 5, and 6 (pan-genotypic). VEL was evaluated with SOF as a fixed-dose combination (FDC) tablet (SOF 400 mg/VEL 100mg) taken by mouth once daily for 12 weeks in four clinical trials (ASTRAL-1, 2, 3, and 5) which included participants with HCV genotype 1, 2, 3, 4, 5, and 6 with HIV co-infection (ASTRAL-5) and among HCV mono-infection (ASTRAL 1, 2, 3) with and without compensated cirrhosis. The regimen evaluated was interferon- and RBV-free. Of note, ASTRAL-4 tested SOF/VEL in participants with decompensated cirrhosis (Child-Turcotte-Pugh classification B, CTP B); patients with CTP B or C are excluded from the present study. Overall, the efficacy of SOF/VEL was high with SVR12 observed in 95-99% of participants (Table 1). In ASTRAL 1, 2, and 3, no HCV relapse was observed between the post-treatment week 12 (SVR12) and post-treatment week 24 (SVR24) which is consistent with HCV eradication or cure. In the ASTRAL-1 (HCV genotype 1, 2, 4, 5, 6) study, SOF/VEL was compared to placebo tablets (delayed treatment group) in a double-blind manner. Importantly, no difference in safety and tolerability was observed between SOF/VEL and placebo with respect to the quantity and quality of reported AEs and observed laboratory abnormalities in ASTRAL 1 that compared SOF/VEL to a placebo. In the ASTRAL-2 (HCV genotype 2) and 3 (HCV genotype 3) studies, SOF/VEL was compared to SOF + RBV in randomized controlled trials. SOF/VEL was superior to SOF + RBV with respect to SVR12 (efficacy) as well as safety

and tolerability. In pooled analyses of data of participants from ASTRAL 1, 2 and 3, the overall efficacy was estimated at 98%.

Table 1. Summary of SVR results from ASTRAL-1, 2, 3 and 5

	SVR 12 (95 confidence interval)			
	ASTRAL-1	ASTRAL-2	ASTRAL-3	ASTRAL-5*
N	624	266	552	106
Overall	99 (98, >99)	99 (96, 100)	95 (92, 98)	95
Genotype				
1a	98.1 (95.2, 100)			95
1b	98.5 (96.5, 99.5)			92
2	100 (96.5, 100)	99 (96, 100)		100
3				92
3a			95.5 (90.9, 100)	
3b			100 (15.8, 100)	
3h			0	
3k			100 (2.5, 100)	
3 (no subtype confirmed)			88.9 (51.8, 99.7)	
4	100 (96.9, 100)			100
5	97.2 (85.1, 99.9)			
6	100 (91.4, 100)			
Disease				
No cirrhosis	99 (97.7, 99.7)		97 (93.5, 98.9)	94
Cirrhosis	99.2 (95.5, 100)		91.3 (82.8, 96.4)	100
Prior Treatment				
Naive	98.8 (97.3, 99.7)		97.1 (93.8, 98.9)	93
Experienced	99.5 (97.3, 99.6)		90.1 (80.7, 95.9)	97

In the United States and Europe, this regimen is commercially available as a fixed-dose combination tablet that includes the nucleotide HCV NS5B inhibitor, sofosbuvir 400 mg and the HCV NS5A inhibitor, velpatasvir 100 mg. For persons with compensated liver disease, this combination is approved in the United States for the treatment of chronic HCV genotype 1, 2, 3, 4, 5, and 6 as a single tablet taken by mouth once daily with or without food for 12 weeks. The brand name of this combination product is Epclusa®.

Sofosbuvir/Velpatasvir/Voxilaprevir (SOF/VEL/VOX). Voxilaprevir, previously known as GS-9857, is an inhibitor of HCV NS3/4A protease. In vitro, sofosbuvir, VEL, and VOX alone displayed potent inhibition against HCV genotype 1a, 1b, 2a, 2b, 3a, 4a, 5a, 6a, and 6e, and the combination of SOF and VOX SOF and VEL, or VOX and VEL demonstrated additive to slightly synergistic activity across multiple genotypes. In vitro cross-resistance studies showed no cross resistance of SOF, VEL, and VOX. SOF/VEL/VOX is therefore expected to have an improved resistance profile compared with the individual agents. This three DAA combination has been evaluated in phase 2 clinical trials and larger phase 3 clinical trials are underway. The contribution of the HCV NS3 protease inhibitor, VOX, to the hepatic clearance of HCV is of particular interest in that the addition

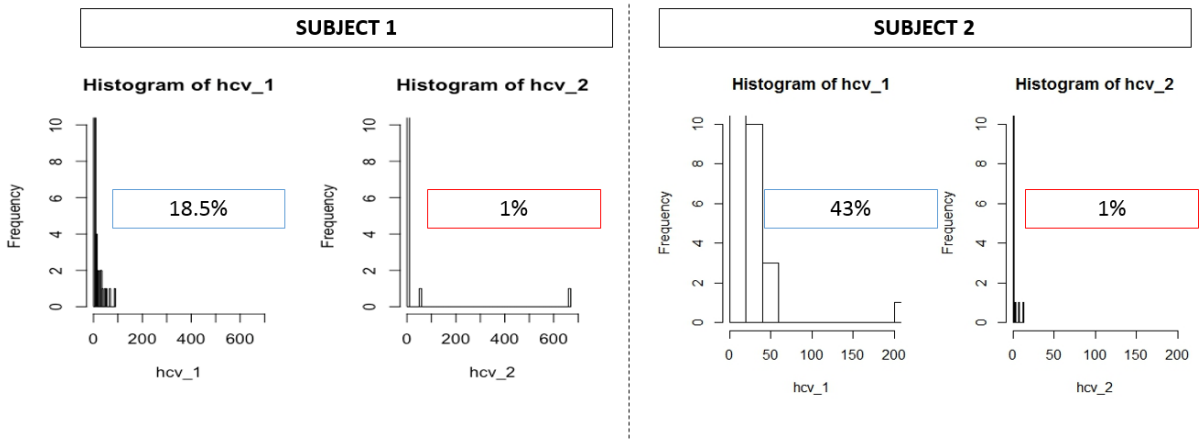
of a DAA with a different mechanism of action to SOF/VEL may prevent to persistence of viral variants with resistance associated polymorphisms which may confer decreased susceptibility to the HCV NS5A inhibitor, VEL and/or the NS5B inhibitor, SOF.

In addition, inhibition of the HCV protease may restore innate intra-hepatic immune functions that are thought to be impaired by the virus. In response to viral replication intermediates, such as viral RNA and proteins, IPS-1 and TRIF adaptor protein act as signaling intermediates in retinoic acid-inducible gene (RIG-I) and/or Toll-like receptor 3 (TLR3) pathways. Activation of these pathways induces IRF3 activation and subsequent transcriptional activation of type I interferons (IFN-α/β) and interferon-sensitive genes (ISGs). Type I interferons and ISGs play a major role in combating viral infections. Cleavage of IPS-1 and TRIF by HCV NS3/4A blocks the downstream signaling pathway, resulting in inefficient activation of IRF3 and severely reducing the host innate immune response against the viral infection. It is possible that HCV protease inhibitors (PI) can play a dual role in countering viral infection through a direct antiviral mechanism, as well as by abrogating the HCV protease-mediated downregulation of innate immunity pathways, such as the RIG-I and TLR3 pathways.

The comparison of the intra-hepatic HCV kinetic and immunologic response after 7 days for dosing with SOF/VEL and SOF/VEL/VOX will permit the in vivo assessment of the role of protease inhibition on hepatic clearance of HCV during DAA treatment.

The change in the timing of the second biopsy is based on investigator review of unpublished, preliminary data from A5335s was presented at the Annual ACTG Network Meeting in June 2017. In this study, two participants with HIV/HCV coinfection underwent paired liver biopsies at day 0 and day 7 of treatment with paritaprevir/ritonavir/ombitasvir + dasabuvir. In both participants, the proportion of HCV-infected hepatocytes had decreased to only 1% (Figure 1). The rationale for the second liver biopsy at day 4 is that this earlier time point will allow better understanding of this rapid and profound reduction is HCV burden and allow for the more precise modeling of HCV elimination from the liver.

Figure 1: Proportion of HCV-infected hepatocytes at Day 0 and Day 7, A5335S



The vertical axis represents the number of HCV-infected hepatocytes counted by sLCM and the horizontal axis represents the HCV RNA production of each hepatocyte in IU/mL (one IU = two copies of HCV RNA) with zero (0) representing an uninfected hepatocyte.

4.0 Study population

A total of 15 adult participants with HCV genotype 1a infection, no prior HCV treatment experience and no evidence of cirrhosis will be enrolled including 10 patients with HCV genotype 1a only (no HIV coinfection) and 5 patients with HIV/HCV genotype 1a coinfection. HIV coinfected patients will be required to be taking protocol specified antiretroviral therapy.

5.1 Inclusion/Exclusion criteria

Inclusion Criteria

Participants must meet all of the following inclusion criteria to be eligible for participation

1. Ability and willingness of participant to provide written informed consent.
2. Men and women age ≥18 to ≤70 years at study entry
3. Body mass index (BMI) ≥ 18 kg/m^2
4. HCV RNA ≥ 10,000 IU/mL at Screening
5. HCV genotype 1a at Screening or within 6 months of screening
6. Chronic HCV infection (≥ 6 months) documented by prior medical history
7. HCV treatment-naïve with no prior treatment with any IFN, RBV, or approved or experimental HCV-specific DAA
8. Absence of cirrhosis as defined as transient elastography (FibroScan®) liver stiffness measurement < 12.5 kPa within 6 months of screening
9. The following laboratory values obtained within 42 days prior to study entry.

- Hemoglobin > 10 g/dL for men and > 9 g/dL for women
- Platelet count ≥90,000/mm^3
- International normalized ratio (INR) ≤1.5
- Calculated creatinine clearance (CrCl) ≥ 30 mL/min
- Alanine aminotransferase (ALT) and aspartate aminotransferase level ≤ 10 x upper limit of the normal range (ULN)
- Total bilirubin <3 mg/dL
- Albumin ≥3.5 g/dL
- CD4+ cell count ≥200 cells/uL and CD4+ cell percentage ≥14% within 42 days of study entry at any US laboratory that has a Clinical Laboratory Improvement Amendments (CLIA) certification [HIV seropositive participants only]
- HIV RNA < 400 copies/mL prior to study entry by any US laboratory that has a CLIA certification or its equivalent [HIV seropositive participants only]

10. On a qualifying antiretroviral therapy (ART) regimen which is permitted with SOF/VEL. This allows for antiretroviral regimen that does not include Efavirenz, Nevirapine, or Tipranavir.

11. Women of childbearing potential must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on Day 0 prior to liver biopsy
12. All participants must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization).

13. If participating in sexual activity that could lead to pregnancy, the participant (men and women) must also agree to use two reliable methods of contraception simultaneously while receiving study treatment and for 30 days after stopping study treatment.

A combination of TWO of the following contraceptives MUST be used appropriately:

- Condoms (male or female) with or without a spermicidal agent
- Diaphragm or cervical cap with spermicide
- IUD (intrauterine device)

14. Participants who are not of reproductive potential (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraceptives. Acceptable documentation of sterilization and menopause is specified below.

Written or oral documentation communicated by clinician or clinician's staff of one of the following:

- Physician report/letter
- Laboratory report of azoospermia
- Follicle stimulating hormone-release factor (FSH) measurement elevated into the menopausal range as established by the reporting laboratory.

15. Participants must be able to adhere to dosing instructions for study drug administration and able to complete the study schedule of assessments, in the opinion of the investigator.

Exclusion Criteria

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

1. Breastfeeding.

2. Known allergy/sensitivity or any hypersensitivity to components of study drugs or their formulation.

3. Acute or serious illness requiring systemic treatment and/or hospitalization within 42 days prior to study entry.

4. Active hepatitis B infection (positive HBsAg) within 42 days prior to study entry.

5. History of decompensated liver disease (including but not limited to encephalopathy, variceal bleeding, or ascites) prior to study entry.

6. Any cause of liver disease other than chronic HCV infection, including but not limited to the following:

- Hemochromatosis
- Alpha-1 antitrypsin deficiency
- Wilson's disease
- Autoimmune hepatitis
- Alcoholic liver disease
- Drug-related liver disease

7. Uncontrolled or active depression or other psychiatric disorder within 24 weeks prior to study entry that in the opinion of the investigator might preclude adherence to study requirements.

8. Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.

9. Serious illness including uncontrolled seizure disorders, active coronary artery disease within 24 weeks prior to study entry, or other chronic medical conditions that in the opinion of the investigator might preclude completion of the protocol.

10. Presence of active or acute AIDS-defining opportunistic infections within 12 weeks prior to study entry.

11. Active or history of malignancy within 2 years prior to study entry other than basal cell carcinoma of the skin and/or cutaneous Kaposi's sarcoma (KS) and/or cervical or anal dysplasia or carcinoma in situ.

13. Infection with any HCV genotype other than genotype 1a, or mixed genotype infection any time prior to study entry.

14. History of major organ transplantation with an existing functional graft any time prior to study entry.

15. History of acquired or hereditary bleeding disorder (e.g., hemophilia, warfarin use) or any other cause of or tendency toward excessive bleeding time prior to study entry.

16. Gastrointestinal disorder or post-operative condition that could interfere with the absorption of the study drug

17. Difficulty with blood collection and/or poor venous access for the purposes of phlebotomy

18. Planning to take any of the following medications or supplements from Day -7 to the end of treatment:

a. Proton pump inhibitors (patients may switch to H2 blockers up to Day -7)

b. Inducers of P-gp (including, but not limited to, dexamethasone, morphine, ritonavir, saquinavir, tipranavir)

c. Moderate to potent inducers of CYP2B6, CYP2C8, or CYP3A4 (including, but not limited to, efavirenz, etavirine, modafinil, rifampin, St. John's Wort, carbamazepine, phenytoin)

19. History of taking any dose of amiodarone within 6 months (180 days) of Day 0

6.0 Study treatment

6.1 **Sofosbuvir/Velpatasvir/Voxilaprevir (SOF/VEL/VOX)**

SOF/VEL/VOX is manufactured as a 400/100/100 mg tablet for oral administration once daily. Participants will take 1 tablet by mouth daily with food. This drug combination includes inhibitors of HCV non-structural proteins (NS) 5B (Sofosbuvir), 5A (Velpatasvir) and 3/4 (Voxilaprevir). Voxilaprevir and the combination of this agent with Sofosbuvir/Velpatasvir is approved by the FDA

and marketed in the United States under the trade name Vosevi ®. Additional information regarding this investigational product can be found in the Package Insert.

Participants may take H2-blockers only when they are taken ≥ 4 hours apart from dosing of SOF/VEL/VOX.

6.2 Sofosbuvir/Velpatasvir (SOF/VEL)

SOF/VEL is manufactured as a 400/100 mg tablet for oral administration once daily. Participants will take 1 tablet by mouth daily without regard to food. This drug combination includes inhibitors of HCV non-structural proteins (NS) 5B (Sofosbuvir) and 5A (Velpatasvir) and is approved by the FDA and marketed in the United States under the name EPCLUSA®. Additional information regarding this commercial product can be found in the Package Insert.

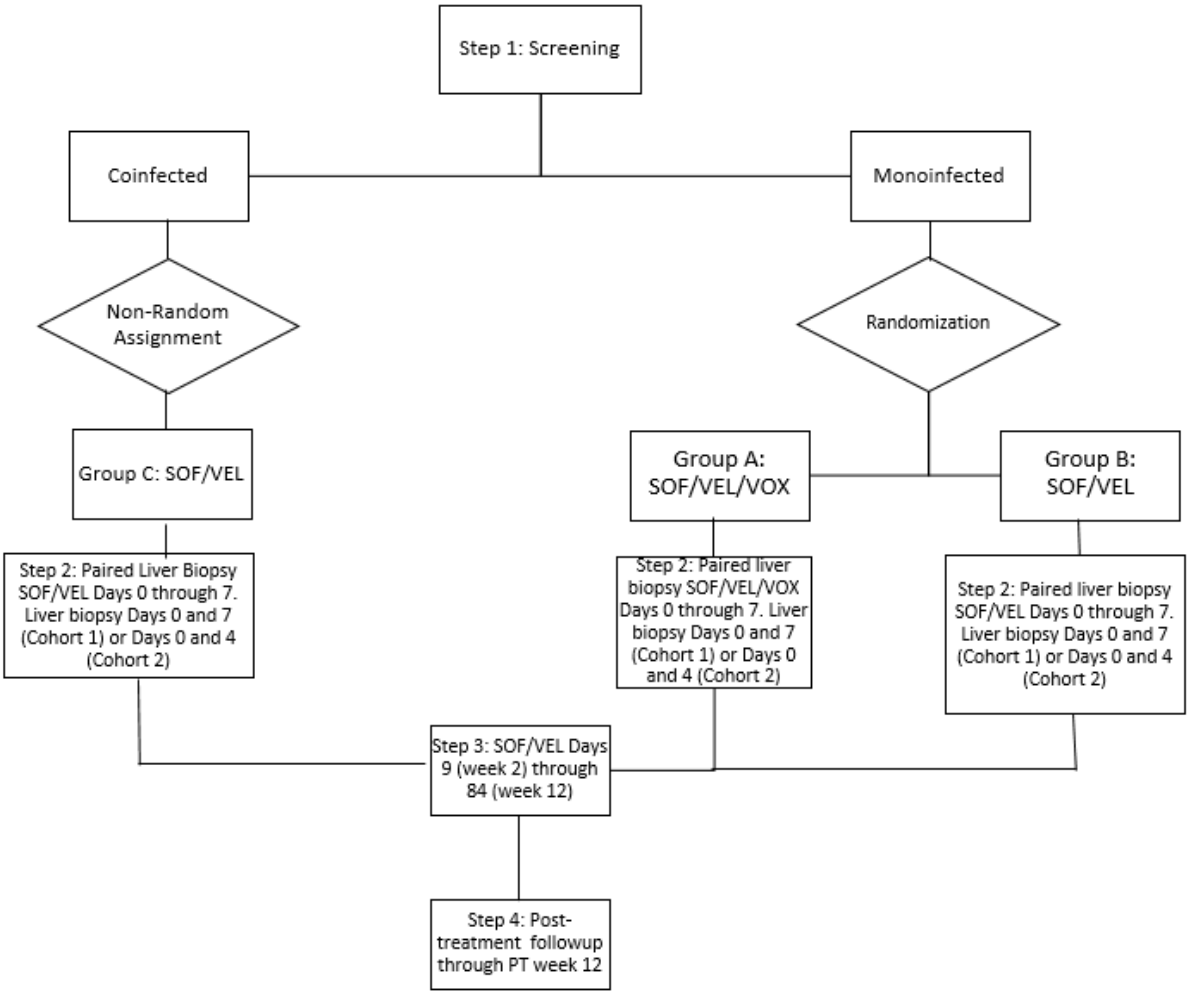
Participants may take H2-blockers only when they are taken ≥ 4 hours apart from dosing of SOF/VEL.

7.0 Study design and Schedule of Events

This study is an open-label, partially randomized clinical trial.

Table 2: Protocol Schema

All subjects will receive a total of 12 weeks of HCV treatment with oral direct acting antiviral (DAA) therapy. The study will occur in four steps.



Cohort 1: Paired liver biopsies on Day 0 and Day 7

Antiretroviral therapy (HIV seropositive only)													
Step 1	Step 2								Step 3			Step 4	
	SOF/VEL/VOX or SOF/VEL								SOF/VEL			Follow-up	
	Entry/D0	D1	D2	D3	D4	D5	D6	D7	WK4	WK8	WK12	WK16	WK24
Screening/ Randomization	Liver BX	VK	VK	VK	VK			Liver BX	VK	VK	VK	VK	VK
	VK							VK					

Cohort 2: Paired liver biopsies on Day 0 and Day 4

Antiretroviral therapy (HIV seropositive only)													
Step 1	Step 2								Step 3			Step 4	
	SOF/VEL/VOX or SOF/VEL								SOF/VEL			Follow-up	
	Entry/D0	D1	D2	D3	D4	D5	D6	D7	WK4	WK8	WK12	WK16	WK24
Screening/ Randomization	Liver BX	VK	VK	VK	Liver BX			VK	VK	VK	VK	VK	VK
	VK												

7.1 **Step 1. Screening/ Randomization**

Following informed written consent, participants will undergo screening procedures as per schedule of Events (Table 4).

The first nine subjects enrolled will be assigned to Cohort 1 as follows: 3 subjects in Arm A, 3 subjects in Arm B, and 3 subjects in Arm C. The subsequent six subjects will be enrolled into Cohort 2 as follows: 2 subjects in Arm A, 2 subjects in Arm B, and 2 subjects in Arm C.

Eligible participants who are coinfected will be randomized before Entry/ Day 0. Randomization will occur for eligible monoinfected subjects with a 1:1 between SOF/VEL and SOF/VEL/VOX. Eligible subjects who are coinfected will be non-randomly assigned to receive SOF/VEL. Subjects must enter Step 2 within 42 days of screening. Screening evaluations to determine eligibility must be completed within 42 days prior to entry, with the exception of IL28B, which can be pulled from historical records or performed during screening, and HCV genotype, which can be pulled from historical records up to 6 months before screening or performed at screening. Subjects may be rescreened at the PI's discretion. Laboratory parameters may be repeated as clinically necessary to be resulted within 30 days of Day 0 as required by Johns Hopkins.

7.2 **Step 2. Paired liver biopsy for intrahepatic HCV dynamics (VK)**

The first 7 days of therapy (one week) represents the primary study assessment in which participants will undergo liver biopsy on treatment day 1 and treatment day 7 (cohort 1) or treatment day 4 (cohort 2) to assess intra-hepatic HCV dynamics and DAA pharmacokinetics.

HIV/HCV monoinfected, eligible participants will be randomly assigned to treatment with Sofosbuvir/Velpatasvir (n=5) or Sofosbuvir/Velpatasvir/Voxilaprevir (n=5) for 7 days. HCV coinfected patients (n=5) will be treated with Sofosbuvir/Velpatasvir for 7 days. As such, there will be three groups:

- Group A. HCV genotype 1a infection; Sofosbuvir/Velpatasvir/Voxilaprevir (SOF/VEL/VOX)
- Group B. HCV genotype 1a infection; Sofosbuvir/Velpatasvir (SOF/VEL)
- Group C. HIV/HCV genotype 1a infection; Sofosbuvir/Velpatasvir (SOF/VEL)

Entry will occur on day 0; entry/day 0 is meant to coincide and be dictated by start of DAA treatment. Upon entry/day 0, participants will be admitted to the Johns Hopkins Hospital clinical research unit and undergo procedures and evaluations as per Table 5. All participants will undergo a core needle biopsy (CNB1), then upon return to the inpatient unit, given a meal and be given their first dose of DAA treatment. The first dose of DAA therapy will be directly observed and followed by plasma HCV RNA monitoring after dosing. Participants will be ordered Acetaminophen and/or Ketorolac as clinically appropriate for the treatment of post-procedure pain while inpatient. The participant will be discharged at approximately 24 hours after the first dose of DAAs on day 1 and followed daily with directly observed dosing in the outpatient setting and on days 2, 3, and 4 with phlebotomy for HCV RNA monitoring. On the 7th day of DAA therapy (cohort 1) or 4th day of DAA therapy (cohort 2), participants will undergo a second core needle biopsy (CNB2), with dosing of study drug immediately following CNB2. This will be performed in the outpatient setting followed by phlebotomy for HCV RNA and discharge to home. Subjects in cohort 2 will return for directly observed DAA dosing immediately after phlebotomy for HCV RNA. Completion of day 7 evaluations will complete participation in Step 2 and participants will enter Step 3 of the study.

Visits during Step 2 must be scheduled on the exact day planned with no visit window. Entry/day 0 and day 7 are to be scheduled 7 days apart. If a participant misses a visit on days 1-6, they will continue on study. Missing a visit does not extend the total duration.

Table 4: Step 1 and Step 2 Schedule of Events

Evaluation	Screening Day -42 – Day -1	Entry/ Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Vital Signs	X	X				X[b]			X[a]
Height	X								
Weight	X								
Documentation of HIV status	X								
Documentation of chronic HCV genotype 1a Infection	X								
Documentation of the absence of cirrhosis by Fibroscan	X								
Medical History	X								
Documentation of current medications	X								
Complete Physical Exam	X								
Comprehensive metabolic panel (calculated creatinine clearance)	X								
CBC	X				X[b]	X[a]			

Evaluation	Screening Day -42 – Day -1	Entry/ Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
INR and PTT	X								
Pregnancy Testing	X								
HBsAg	X								
CD4+ (HIV seropositive only)	X								
HIV RNA (HIV seropositive only)	X								
IL28B genotype	X[c]								
HCV Genotype	X[d]								
DAA dosing		X	X	X	X	X	X	X	X
Plasma Viral Kinetics (HCV RNA)	X	X	X	X	X	X			X
Stored PBMCs (Immunology)		X				X[b]			X[a]
Stored Plasma (Resistance)		X	X	X	X	X			X
Core Needle Biopsy (Liver)		X				X[b]			X[a]
Post-Biopsy Clinical Evaluations		X				X[b]			X[a]

[a] Evaluations to be performed at these timepoints for cohort 1 only

[b] Evaluations to be performed at these timepoints for cohort 2 only

[c] IL28B result can be pulled from historical records with no time restriction prior to screening or performed at screening if a prior result is unavailable.

[d] HCV Genotype result can be pulled from historical records within 6 months of screening. or performed at screening if a prior result is unavailable.

7.3 **Step 3. HCV treatment with SOF/VEL**

The remaining 11 weeks of DAA therapy represents standard of care treatment for HCV.

Following the completion of Step 3, all participants will be given treatment with sofosbuvir/velpatasvir fixed-dose combination tablet from treatment day 8 to day 84 (total treatment duration of Step 2 plus Step 3 is 84 days or 12 weeks). Plasma HCV RNA levels will be obtained as per Table 5. Visits must occur within the windows indicated on the schedule of events.

7.4 **Step 4. Post-treatment follow-up**

After completion of HCV treatment, all participants will have study visits at weeks 16 and 24 with evaluations as per Table 5. Visits must occur within the windows indicated on the schedule of events.

Total study duration: Up to 30 weeks.

Table 5: Step 3 and Step 4 Schedule of Events

	Step 3 HCV Treatment with SOF/VEL				Step 4 Post-treatment Follow Up	
Evaluation	**Week 2 ± 3 days**	**Week 4 ± 3 days**	**Week 8 ± 3 days**	**Week 12 ± 3 days**	**Week 16 ± 5 days**	**Week 24 ± 5 days**
Plasma Viral Kinetics (HCV RNA)	X	X	X	X	X	X
Concomitant Medication	X	X	X	X		X
Targeted Physical Exam				X		X
Stored PBMCs (Immunology)		X				X
Stored Plasma (Resistance)	X	X	X	X	X	X

8.0 Materials and Methods

8.1 Dosing

All doses of study treatment should be taken at or around the same time of day. During Step 2, subjects should not take their dose of study drug until after each pre-dose sample is obtained on VK days. Once the VK is obtained, each dose of study drugs will be taken orally with approximately 240 mL of water approximately 30 minutes after a meal or snack for Groups A, B, and C.

8.2 Dietary Requirements

Subjects will fast as directed by the radiology department on Entry/ Day 0 in preparation for CNB1. Upon return to the clinical research unit after CNB1, subjects receive a standardized diet on entry/day 0 that will provide approximately 40% of the daily calories from fat and up to 45% of the daily calories from carbohydrates (approximately 2200 calories/day). Snacks may be given ad lib with no restrictions on Day 0. On days 1-6, participants will have a diet ad lib.

8.3 Liver Fibrosis Staging

A sample of the core liver biopsy tissue from CNB1 will be sent for clinical evaluation of liver disease stage by the liver pathologist at the site (clinical care). Handling of this sample will occur as per Johns Hopkins Standard Operating Procedures (SOPs).

8.4 Plasma Viral Kinetics (VK)

Plasma for VK will be collected for HCV RNA measurements at entry/day 0 (intensive sampling) and days 1, 2, 3, 4, and 7.

Upon admission to the inpatient research unit on Day 0, a peripheral IV will be placed or phlebotomy performed and the predose VK sample will be collected. After CNB1 is performed on entry/day 0, participants will be immediately returned to the inpatient unit and will receive a standardized meal. About 30 minutes after the meal is given, study drug will be administered. HCV RNA and plasma immunology measurements will be timed based on DAA administration, with DAA administration being considered Hour 0 for the VK portion of the study. Thereafter, intensive VK sampling over 24 hours will be conducted, with post-dose samples being collected at 4h, 6h, 12h, 18h, and 24h after drug administration.

After the 24-hour intensive plasma VK measurements are completed (on day 1), participants with be administered DAAs for day 1 and discharged to home. Thereafter, they will return daily for phlebotomy on days 2, 3, and 4 timed in relation to the intensive plasma VK samples performed on entry/day 0. Samples will be timed so that they are obtained 24 ± 2 hours from the first administered dose of study drugs on entry/day 0 if this is possible based on the subject schedule. Phlebotomy will be performed for plasma VK (HCV RNA) measurements and plasma immunology measurements. DAA will be administered after phlebotomy on days 2, 3, and 4.

On day 7, participants will undergo phlebotomy for plasma VK (HCV RNA) measurements and plasma immunology measurements.

Table 6: 24 Hour Intensive VK Specimen schedule for entry/Day 0 and Day 1 *(sample)*

Study Assessment or Procedure	Expected Time	
	Day 0	Day 1
Admission to Osler 5	6:00	-
Place peripheral IV	6:15	
Collect predose blood sample	7:30	
Ambulatory or transport to liver biopsy	8:00	
Liver biopsy (CNB1)	9:00	
Vital signs and post-procedure site assessment- 0 hour post biopsy	9:30	
Stretcher transport return to Osler 5	10:00	
Administer standardized meal	10:30	
Vital signs and nursing assessment- 1 hour post biopsy	10:30	
Dosing of study drug with 240 mL of water 30 minutes after meal	11:00	
Vital signs and nursing assessment- 2 hour post biopsy	11:30	
Snack ad lib	11:00-15:00	
Collect 4 hr post-dose blood sample	15:00	
Administer standardized meal after 4 hr post-dose	15:15	

Collect 6 hr post-dose blood sample	17:00	
Administer standardized meal after 6 hr blood sample and before 12 hr blood sample	17:00-23:00	
Collect 12 hr post-dose blood sample	23:00	
Snack ad lib		00:00-10:30
Collect 18 hr post-dose blood sample		5:00
Administer standardized breakfast		10:30
Collect 24 hr post-dose blood sample		11:00
Dosing of study drug with 240 mL of water		11:00
Remove peripheral IV		11:15
Discharge subject from CRU		12:00

8.5 Stored PBMCs (Immunology)

PBMCs for immunology studies will be collected as outlined in the schedule of events. 10 mL of whole blood will be collected in a heparin tube and brought to the research lab for prompt processing.

8.6 Stored Plasma (Resistance)

A single plasma sample will be archived for analysis of viral resistance as outlined in the schedule of events. 10 mL of whole blood will be collected in an EDTA tube and brought to the research lab for prompt processing.

8.7 Core Needle Liver Biopsy

Core liver biopsy 1 will be performed in two passes or more as needed to obtain adequate tissue, and a technician will be present during the harvesting of liver tissue for prompt processing.

Core liver biopsy 2 will then be performed in one pass or more as needed to obtain adequate tissue, and a technician will be present during the harvesting of liver tissue for prompt processing.

8.8 Liver Tissue preparation and intra-hepatic VK analysis

An outline of single cell Laser Capture Microdissection (scLCM)

To address the mechanisms underlying HCV clearance, we will use several novel techniques to measure the number and the distribution of HCV-infected hepatocytes, intracellular immune responses, and intrahepatic drug concentrations. Single hepatocytes will be separately analyzed for their HCV RNA content and innate immune responses by integrating scLCM and quantitative PCR (qPCR). We will employ four major methodological advances to study the clearance rate of HCV-infected hepatocytes during DAA treatment: i) scLCM, ii) high-resolution

qPCR, iii) unbiased sampling with an integrated image bank to map the viroscape, and iv) single-cell HCV Core-E1 sequencing.

Tissue preparation. Liver tissue is processed and placed on glass and polyethylene naphthalate (PEN) membrane slides while being kept at -24ºC for the duration of their pre-visualization time to preserve RNA. After staining, slides are stored at -80ºC until scLCM. RNAse inhibitors such as RNAlater® are avoided as their high viscosity impedes scLCM.

scLCM. A Leica LMD 6500, laser microdissection system, was acquired by Dr. Ashwin Balagopal. The instrument integrates a high-density photon laser with a fully motorized light microscope. Since bulk tissue analysis pools disparate signals, and tissue disruption with cell purification neglects anatomic context, conventional microdissection was developed to enrich cells of a given type for downstream analysis. Previous studies have required hundreds to thousands of cells. We have improved the technique by dissecting individual liver cells into separate containers, developing scLCM through rigorous testing and validation using liver samples from HCV-infected subjects [5, 8].

Hepatocytes are dissected from a 10mm 16-gauge segment of liver using the fully integrated Leica LMD 6500 system. Ten micro-thick tissue segments are cut and placed on a membrane, ethanol-fixed, and quick-stained with hematoxylin to reveal cell nuclei and hepatocyte morphology without compromising RNA recovery. Then, the UV-A diode-laser is focused through the microscope objective to achieve a spot size of less than 1 micron in diameter with the 100x objective. The extremely high photon density in the narrow laser focus creates locally-restricted, cold ablative photolysis without heating. The laser is then used to cut around the cell which drops by gravity into the RNA extraction media in the closely appositioned microcentrifuge collection tube cap. The motorized stage (128 nm stepping) is controlled by integrated Leica circuitry and software. This technique permits high yield specimen retrieval and performance is highly consistent in Dr. Balagopal's laboratory at John Hopkins University. Using scLCM, hepatocytes are isolated in 10x10 grids. Based on previous results, viroscapes are constructed for an average of 5 fields from each subject, totaling 500 hepatocytes, and mapped to a positional matrix (Figure 2). The data generated includes a) the proportion of infected hepatocytes; b) the magnitude of HCV infection per cell (defined as HCV RNA IU/cell [viral RNA (vRNA)]); and c) the number and depth of infected hepatocyte clusters.

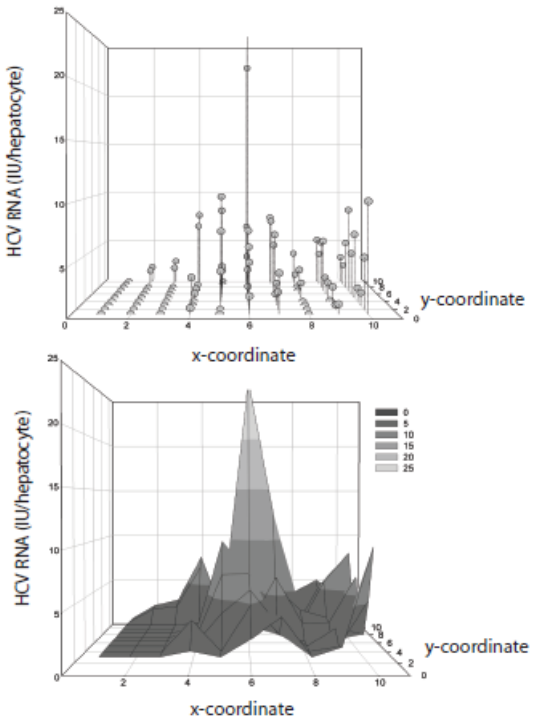


Figure 2. Representative HCV viroscape.

HCV vRNA was quantified in a grid of 10X10 hepatocyte captures and normalized to a known standard. A map was assembled; x- and y- axes depict spatial coordinates using arbitrary units, and the amount of normalized HCV vRNA is

High-resolution qPCR. After capture and immediate analysis, RNA extraction is performed with the Agencourt® RNAdvance Cell v2 system that uses paramagnetic bead separation of nucleic acids, yielding high concentrations of viral and host RNAs. After extraction, specimens are divided into thirds. The first portion is tested for vRNA using the ABBOTT® RealTime HCV Assay that has been adapted for use on tissue samples (Figure 3). The second portion is used to measure 7SL, an abundant and conserved non-coding cytoplasmic RNA, using qPCR, to control for the amount of inputted RNA. 7SL is stable across hepatocytes, preventing skewing of target RNA estimates by variable cytoplasmic capture (Figure 4). Other intracellular RNAs are also quantified using the second portion. The third portion is used for Core-E1 sequencing of the HCV genome.

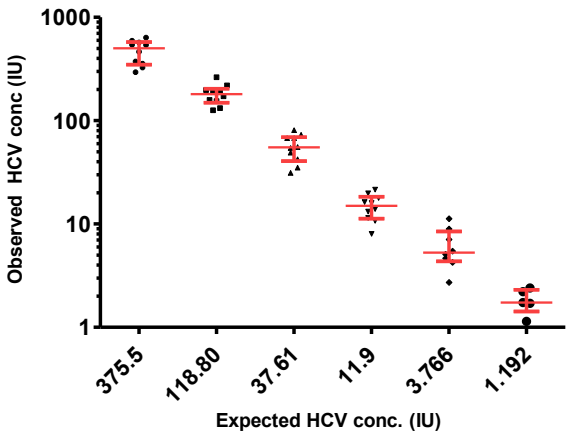


Figure 3. Limit of sensitivity of HCV qPCR assays tested by spiking in known amounts of HCV RNA into RNA extracted from HCV-uninfected tissues that were composed of 100 cells and subsequently serially diluted. Indicated are medians with IQR.

Additional mRNAs will be tested using stored complementary DNA (cDNA) from each captured hepatocyte. In contrast to vRNA, mRNAs often require one round of linear amplification before quantification. The Nugen WT-Ovation One-Direct system uses poly-thymidine and random hexamer priming to provide maximal coverage of coding RNAs, and is used to linearly amplify RNA non-specifically before quantification.By optimizing RNA isolation and purification, and optimizing qPCR protocols, we consistently measure viral and host RNA from ~95% of scLCM samples. We have also developed a protocol for high-resolution reverse trancription whereby we add short-length 5' and 3' adaptors to extracted RNA molecules; we then perform PCR using primers that hybridize to the adaptors, amplifying a cDNA library non-specifically from single cells. Quantitative PCR (qPCR) is performed on the library using primers and probes that are specific for target mRNAs.

High-resolution qPCR permits interrogation of the same cells for multiple pieces of data and, importantly, allows for discarding of suboptimal data (defined by insufficient amounts of a housekeeping RNA). *In situ* scLCM has the advantage of finding natural and consequently relevant determinants of HCV replication. While other methods exist to enumerate HCV-infected hepatocytes, we are not aware of another team using LCM to quantify the burden of infection per cell, nor of comparing host RNAs in infected and uninfected cells.

Unbiased sampling and integrated image bank mapping. Initial attempts to identify and quantify HCV-infected hepatocytes *in situ* were based on prior immunohistochemical (IHC) staining for HCV proteins, followed by dissection of HCV+ hepatocytes. These attempts were impeded by inconsistent staining, hepatic autofluorescence, and RNA loss under conventional staining protocols. We have circumvented traditional approaches by separately isolating consecutive hepatocytes prior to determining whether they are infected [5]. Pre- and post-scLCM images are stored on a secure server. After high-resolution qPCR of coded scLCM samples, virtual maps of HCV infection are assembled using stored images. The advantage of this approach is that it leverages quantitative methods, rather than competing in situ methods, which are semi-quantitative or even qualitative, to reveal the peaks and nadirs of HCV replication in the liver, which we have termed the viral landscape, or viroscape. The quantitative viroscape is optimally conceived for modeling applications where qualitative approaches would be inadequate. The sampling is spatially unbiased, in that hepatocytes are collected consecutively in antiparallel grids, irrespective of whether they are infected. Infection of a given hepatocyte is only determined after processing each cell separately, and operators are blinded to positional maps until final analysis. Since the minority of hepatocytes is HCV-infected, unbiased sampling permits comparisons of intracellular signals between infected hepatocytes and adjacent uninfected hepatocytes that are presumably refractory to infection despite high HCV viral loads. A linked advantage to this approach is that, rather than committing full tissue sections to interrogation of a single or small number of host mRNA molecules, each scLCM-dissected cell can be separately interrogated for multiple signals.

A key feature of the Leica LMD 6500 is that full section images are saved at low and high resolution before and after laser capture. After viral and host RNA measurements are complete,

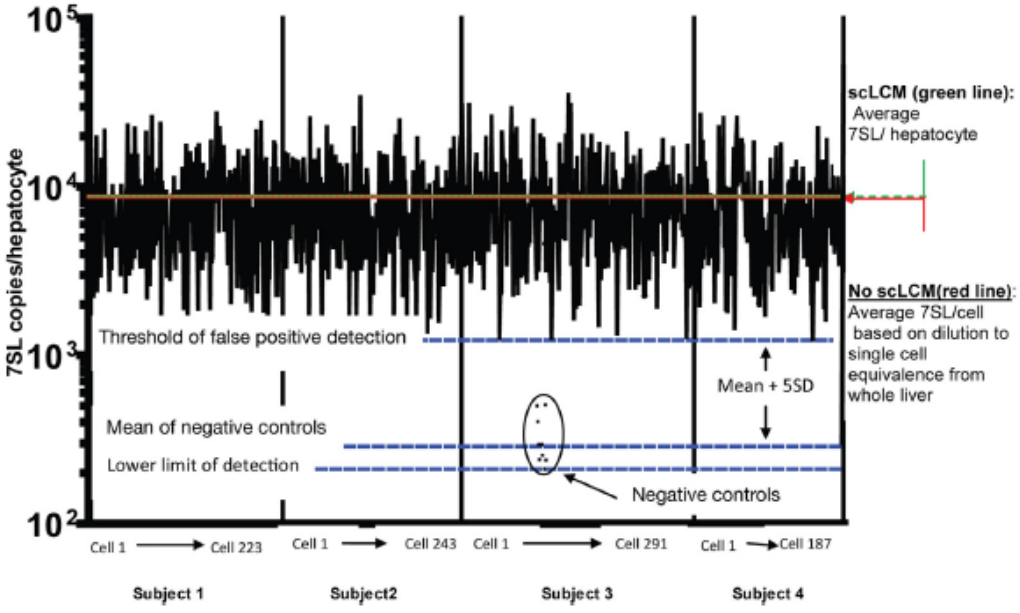


Figure 4. RNA isolation from scLCM is consistent, and results in negligible RNA loss. qPCR in each scLCM-isolated hepatocyte against a quantitative standard (mean value is indicated by *hashed green line*) and was found to be comparable with RNA from single-cell equivalents of homogenized bulk liver tissue that was isolated by the same technique but without exposure to the laser (mean value is indicated by the *red line*). Single-cell equivalency of bulk liver homogenates was estimated by qPCR measurement of ERV3 DNA, which is an endogenous retroviral element for which two copies are found in all mammalian cells. Negative control empty isolations of equivalent volume of polyethylene naphthalate membrane were derived from the same slides and found to contain low levels of 7SL (*black dots)*. A stringent threshold of 5 standard deviations over the mean negative control amount was set to avoid false quantification of cell fragments.

a positional matrix corresponding to the 10x10 dissection space is created and populated with RNA quantities. In addition, physical distances from each cell will be plotted to major hepatic structures (eg, portal tracts and centrilobular veins) that define lobular anatomy to account for differential intracellular drug concentrations and metabolism based on hepatic blood flow and lobular zones.

Single-cell HCV Core-E1 sequencing. The Core-E1 sequencing amplicon and cloning system is used to reliably study the diversity and evolution of the HCV quasispecies in plasma samples. The conventional Core-E1 assay uses amplification from nested PCR reactions that yield a final product of 473 bp. An advantage of this system is that it consistently produces interpretable results – the inclusion of E1 permits sufficient variability that phylogenetic distances can be calculated within subject and indeed even within a cluster of infected hepatocytes. We have adapted sensitive sequencing of the HCV Core-E1 regions from plasma to scLCM samples from tissue, and have successfully obtained sequence from hepatocytes with as low as 3 IU/cell. Core-E1 sequencing can be used to validate by an independent technique that a given cell is indeed infected. In addition, while previous studies have relied on bulk sequencing of HCV from bulk tissue, no information from such studies could be used to discriminate mechanisms of clearance of infected hepatocytes, since the resolution would be limited to the smallest unit studied. An added advantage of single-cell sequencing, through the design of separate amplicons that target different regions of the HCV genome, is in detecting drug resistant viral variants before and during DAA therapy in individual cells that may not be apparent from sequencing of the plasma quasispecies. Although no resistance is expected, core-E1 sequencing on 2-3 hepatocytes per 10x10 grid with high-level vRNA can be performed and compared between biopsies. (Sequencing all infected hepatocytes in the viroscape could be more informative, but this is beyond the resources of this study).

Intrahepatic HCV infection was studied intensively using scLCM and spatial statistical analysis in liver tissue from four subjects of the ALIVE study of injection drug users (IDUs) who had chronic genotype 1 infection. By dissecting > 1000 individual hepatocytes, the viroscape was uncovered in eleven separate grids (Figure 2), demonstrating clusters of HCV+ hepatocytes; 21-45% of all hepatocytes were infected. HCV vRNA was measured in each hepatocyte and ranged from 1 – 50 IU across all subjects with the majority of cells producing < 10 IU. This technology has been extended to allow HCV sequencing from individual hepatocytes. Moreover, IFN responses were measured in the same hepatocytes; in contrast to clustered HCV infection, a representative ISG, IFITM3, was found to have a scattered expression pattern that was neither directed toward nor away from HCV clusters. Indeed, few HCV+ hepatocytes expressed the ISG. These results suggest poor targeting of ISG expression to HCV-infected hepatocytes, and may partly explain persistence of HCV infection. Since HCV RNA amounts are the principal measures of effective DAA treatment, scLCM with high-resolution qPCR and spatial statistical analyses form a highly innovative approach to the study of intrahepatic HCV infection before and during HCV DAAs and to study the effect of HIV coinfection on the host response.

8.9 Post-Biopsy Clinical Evaluations

The post-biopsy clinical evaluations will consist of vital signs and an assessment of pain at the biopsy site. Evaluations for excessive pain or bleeding will be performed within the first 4 hours after CNB1 and CNB2 by the nursing staff in the CTRC. Pain and bleeding will be principally

assessed clinically by questioning and using vital signs (heart rate and blood pressure), obtained every hour for the first 2 hours after biopsy. Subjects will be monitored for complications from liver biopsy as found on the American Association for the Study of Liver Diseases (AASLD) website: http://www.aasld.org/practiceguidelines/Documents/Bookmarked%20Practice%20Guidelines/Liver%20Biopsy.pdf.

9.0 Adverse events

9.1 Non-serious adverse events

The maximum grade of all adverse events will be collected from day 0 through week 4 and documented on the case report form (CRF). At each visit, adverse effects will be ascertained. All inpatient and outpatient clinical assessments and results will be reviewed for the presence of adverse events. All patients will be evaluated by non-directed questioning for the occurrence of adverse events during the course of the study. After noting any *volunteered* adverse events, the question, "Have you had any other problems since I last saw you?" will be asked at each visit. Adverse events will then be explored through directed questioning. Standard guidelines for the management of toxicity will be closely followed to minimize risk to study participants. Adverse events will be graded according to standard clinical trial guidelines.

9.2 Serious adverse events

Serious adverse events will be collected from time of consent to day 0 and from week 4 to post treatment week 12.

The investigator will notify the Institutional Review Board (IRB) and the FDA of all serious adverse events in writing within 24 hours in accordance with international and local laws and regulations. Serious adverse events are defined by the International Conference on Harmonisation (ICH) as an event that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an other important medical event (may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the events listed above.

10.0 Sample Size

The anticipated sample size for analyses is 15 participants with 5 in each of three distinct groups. Group C will enroll 5 patients with HIV/HCV genotype 1a coinfection and will receive treatment with SOF/VEL representing inhibitors of HCV NS5B and NS5A a. Group A and B will

each enroll 5 patients with HIV genotype 1a monoinfection; Group A will be treated with SOF/VEL/VOX and Group B will receive only SOF/VEL representing inhibitors of HCV NS5B and NS5A only. As such, Groups B and C are treated with same two-drug DAA regimen and will permit the comparison of persons with HIV/HCV and HCV. In contrast, Groups A and B include patients with HCV monoinfection and differ by the use of the HCV NS3/4 inhibitor Voxilaprevir in Group A and will permit the comparison of the NS3/4 inhibition versus none.

Because this study is exploratory and is enrolling a very small sample, traditional statistical power calculations are not being used to justify the sample size. Instead, this sample size was developed based on the feasibility of both anticipated enrollment and the feasibility of the laboratory procedures, namely single cell laser microdissection.

For the primary outcome of difference in proportion of HCV-infected hepatocytes between week 1 and baseline, we present an approximate confidence interval around the difference in proportion within subject to show the level of precision in this exploratory single-arm study. The following assumptions are made to approximate the confidence interval in a conservative way:

- for within-subject variation – proportion of infected hepatocytes at any particular time-point is 50% (this assumption is conservative as results in maximum variation),
- the number of hepatocytes measured within each subject is 400 (this is conservative as it is estimated that between 400 and 1000 hepatocytes will be measured),
- normal approximation to the binomial for standard error estimation, and the variance of the difference will be approximated as twice the variance (assuming independence between week 1 and baseline, which is conservative except under negative correlation, which is not expected given the reported potency of the intervention being studied).

These assumptions give an estimated within-subject standard error of 0.035 for the primary outcome and can be used to formulate a confidence interval on the difference in proportions of HCV-infected hepatocytes between week 1 and baseline on a single individual. For other less conservative assumptions (larger number of hepatocytes sampled, or estimated proportion of HCV-infected hepatocytes being either larger or smaller than 0.5 at either week 1 or week 0), the within subject standard error for the difference in proportions outcome would be smaller than 0.035, and so this represents the maximum standard error for the given assay parameters that specify that at least 400 hepatocytes will be measured in each subject at each time point.

For combining the estimated differences across subjects in order to obtain a study sample estimate for the difference in proportion of HCV positive hepatocytes between week 1 and baseline, the average difference across the 5 subjects within each group will be calculated. Assuming independence across individuals, and using the above maximum within subject standard error for each of the 5 subjects gives a standard error for the average as 0.035/sqrt(5), which is 0.015. This was obtained using conservative assumptions for the purpose of sample size considerations in this exploratory study. Therefore, the estimated maximum half-width of a two-sided 95% confidence interval on the study sample average difference in proportion is approximately 1.96*0.015 = 0.031.

11.0 Statistical Analyses Plan:

All outcomes will be summarized and described with appropriate summary statistics. For example, estimated binomial proportions (e.g., for % of HCV-infected hepatocytes) will include 95% confidence intervals. Means, medians for continuous outcomes will include confidence

intervals and key percentiles (eg, 25th, 75th percentiles), respectively. Outcomes defined as changes will also present summary distribution of values at each time point contributing to change outcome. In addition, because of small sample size, outcomes will also be summarized on a per-subject basis for reporting. Changes will be defined as absolute differences for those outcomes where baseline distribution of proportion is close to 0 or 1, and relative (and absolute secondarily), changes otherwise. For continuous outcomes, both absolute and relative changes from baseline will be explored and summarized. Confidence intervals (using exact methods) on change outcomes will be calculated and presented.

For the primary outcome, the estimated differences will be summarized and described on a per-subject basis (i.e. 15 differences and intra-subject confidence intervals). Additionally, the proportion of infected hepatocytes will be summarized across subjects at each time point and presented. Finally, the average difference from week 0 to week 1 (absolute difference) for the study sample will be estimated and summarized.

For the plasma VK secondary outcome, longitudinal modeling will be used to combine plasma HCV viral loads (VL) over the first week. For instance, marginal modeling will focus on estimation of levels across the study sample (ie, population level inference). This can be used to describe the average change in HCV VL over the first week on treatment. In addition, (generalized linear) mixed effects models will focus on subject-specific inference (ie, prediction). Since this is a small study, these data will be graphically represented by subject.

Associations between two continuous outcomes will be described using rank-based (e.g., Spearman, using exact method versions with PROC STATXACT [specifically PROC ASSOCIAT]) correlations (and will be presented graphically using scatterplots), and associations between a continuous and a categorical outcome will be summarized by separately summarizing distribution of the continuous outcome by the other covariate category (level), as well as testing for differences in the distribution of the continuous covariate by varying levels of the categorical covariate using the exact version of Kruskal-Wallis test (equivalent to the exact version of Wilcoxon rank sum tests for more than two categories). For restricted alternatives of monotonicity among more than two groups, additional statistical tests such as Jonckheere-Terpstra may also be used.

Contingency tables will describe ordinal fibrosis staging (non-continuous outcomes), and distributions of continuous outcomes will be summarized by fibrosis stage, as necessary.

12.0 Data Safety Monitoring

The study will have external oversite by a Johns Hopkins University Study Monitoring Committee (SMC) comprised of two physicians with extensive training in both HIV and HCV disease. The SMC will conduct its first review when 5 participants have completed Step 2 (paired liver biopsy) and, if deemed necessary by the SMC members, may conduct a second interim review after 10 participants have completed Step 2. The SMC will not oversee the conduct of Step 3 since this reflects treatment with an FDA-approved HCV regimen and the conduct of Step 3 and 4 will be under standard of care procedures.

13.0 Regulatory Compliance

12.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. A signed consent form will be obtained from the participant (or legal representative or person with power of attorney for participants who cannot consent for themselves). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject, or legal representative and this fact will be documented in the participant's record.

12.2 Good Clinical Practice

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP), and all applicable regulatory requirements.