

CLINICAL RESEARCH PROTOCOL

NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

DATE: November 25, 2019

CLINICAL PROTOCOL NO.15-DK-0143

IND NO:126497

IND Name: Sofosbuvir/GS-5816

IND HOLDER: NIDDK

TITLE: Investigation Of Viral Kinetics, Interferon Stimulated Genes (ISGs) and mirRNA Among Subjects Infected With Different Hepatitis C Virus Genotypes During Therapy With Sofosbuvir and GS-5816

SHORT TITLE: Once daily all oral therapy for chronic hepatitis C

IDENTIFYING WORDS: Sofosbuvir, GS-5816, Direct Acting Antivirals, Chronic Hepatitis C, Treatment naive, Relapsers, Cirrhosis, Liver Biopsy.

PRINCIPAL INVESTIGATOR: Marc Ghany, M.D., MHSc, Liver Diseases Branch (LDB), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)*

LEAD ASSOCIATE INVESTIGATOR: T. Jake Liang, M.D., Chief, Liver Diseases Branch (LDB), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)*

ASSOCIATE SENIOR INVESTIGATORS:

Barbara Rehermann, M.D., Senior Investigator, LDB, NIDDK

Elizabeth Wright, Ph.D. OD, NIDDK

David Kleiner, M.D., PhD., Laboratory of Clinical Pathology, NCI

Robert Shamburek, M.D., Lipid Section, Translational Medicine Branch, NHLBI

ASSOCIATE STAFF INVESTIGATORS:

Elenita Rivera, R.N., Research Nurse, LDB, NIDDK

*Investigators with this designation are authorized to obtain informed consent for this protocol

ESTIMATED DURATION OF STUDY: 3 Years

NUMBER AND TYPE OF PATIENTS: 140 patients with chronic hepatitis C, ages above 18 years, both male and female

SUBJECTS OF STUDY:

Number of patients: 140
18 years

Sex: Male & Female

Age Range: Above

Volunteers: None

PROJECT USES IONIZING RADIATION: Yes, for medical indications only.

PROJECT USES "DURABLE POWER OF ATTORNEY": No

OFF-SITE PROJECT: No **MULTI-INSTITUTIONAL PROJECT:** No

Table of Contents

Précis	6
A. Background: Chronic Hepatitis C and Current Therapy:	7
B. Hypothesis and Aims:	9
B.1 Hypothesis:	9
B.2 Aims:	9
C. Trial design	9
D. Summary Of Investigational Agents	11
D.1.Sofosbuvir (SOF) (formerly GS-7977)	11
D.2. GS-5816	11
D.3. SOF/GS-5816 Fixed Dose Combination (FDC)	11
E. Protocol	11
F. Study Population	12
F.1. Inclusion Criteria	12
F.2. Exclusion Criteria	14
G. Treatments	16
G.1 Description and Handling of SOF/GS-5816 FDC	16
G.1.1 Formulation	16
G.1.2 Packaging and Labeling.....	16
Sofosbuvir/GS-5816 FDC and placebo-to-match tablets are packaged in white, high-density polyethylene bottles. Each bottle contains 28 tablets and polyester packing material. Each bottle is enclosed with a white, continuous thread, child-resistant screw cap with an induction-sealed, aluminum-faced liner.....	16
G.1.3 Storage and Handling	16
G.1.4 Stability.....	16
G.1.5 Dosage and Administration of SOF/GS-5816 FDC.....	17
G.2.Study Drug Adherence and Drug Accountability	17
G.3.Concomitant Medications	17
H. Study Procedures	18
H.1 Consent Process	18
H.1.2 Short Form Consent.....	19
H.2 Investigator Responsibilities	19
H.3. Pre-treatment Phase: (Screening)	20
H.3.1 Outpatient Screening Visit:.....	20
H.3.2 Ancillary Study procedure	20
H.4 Completion of Screening / Admission for Pre-Treatment Liver Biopsy / Start of Treatment:	21
H.5 Monitoring	22
H.5.1 Weeks 1, 2 & 3, (\pm 3 days):.....	22

H.5.2. Week 4: (\pm 5 days).....	22
H.5.3 Week 8 (\pm 3 days):.....	23
H.5.4 End of Treatment (Week 12 \pm 3 days)).....	23
H.5.5 Ancillary Study Procedure	24
H.6 Post-treatment weeks 2, 4, 8 and 12 (\pm 5 days)	24
H.7 Final study visit week 24 post-treatment (\pm 5 days).....	24
I. Discontinuation of Subjects from Treatment	25
I.1 Virologic Response-Based Treatment Stopping Criteria:	25
I.2 Other Stopping Criteria:.....	25
I.3 Early Termination (ET) Visit.....	26
J. Unscheduled Visits	26
K. Procedures.....	27
K.1 Assays for Monitoring HCV RNA levels and Genotype/Subtype determination:.....	27
K.2 Management of non-response.....	27
K.3 Management of virological breakthrough.....	27
K.4 Medical History.....	27
K.5 Complete Physical Examination.....	27
K.6 Vital Signs	27
K.7 Creatinine Clearance	28
K.8 Body Mass Index (BMI)	28
K.9 12-Lead ECGs	28
K.10 Viral RNA Sequencing / Phenotyping Sample	28
K.11 IL28B Testing.....	28
K.12 Pregnancy Testing	28
K.13 Health Related Quality of Life Surveys	29
K.14 Nuclear Medicine Resonance Lipoprotein Profile	29
L. Hazards and Discomforts	29
L.1 The risks and discomforts of frequent phlebotomy:.....	29
L.2 The risks and discomforts of HIV testing.	29
L.3 The risks and discomfort of percutaneous and transjugular liver biopsy.	30
L.4 The risks and hazards of sofosbuvir therapy.	30
L.5 The risks and hazards of GS-5816 therapy	32
L.5.1 Adverse Events ^[1] _{SEP}	32
L.5.2 Death	33
L.5.3 Serious Adverse Events.....	33
L.5.4 The risks and hazards of sofosbuvir and GS-5816 combination therapy safety conclusions	34
L.6 The risks and hazards of lymphaphoresis.....	35
M. Safety Assessments	35
N. Toxicity Management.....	36

O. Adverse Event Reporting	36
O.1 Reporting to the IRB	36
O.2. Reporting to the FDA	37
O.3 Reporting to Gilead	37
P. Protocol Monitoring Plan	38
Q. Viral Resistance	38
R. Efficacy Assessments	39
R.1 Primary Efficacy Assessment	39
R.2 Secondary Efficacy Assessment	39
R.3 Other Assessments (Endpoint Definitions):	39
S. Statistical analysis	40
S.1 Planned Interim Analysis for Futility:	40
S.2 Demographics and Baseline Characteristics	40
S.3 Analysis plan	40
S.4 Analysis methodology	41
T. Enrollment of Children, Women and Minority Individuals	41
U. Additional Potentially Vulnerable Subject Populations	42
V. Research Use, Storage and Disposition of Human Samples and Data:	42
W. Protocol Modifications	43
References:	44
Appendix I	47
Ancillary Study I	47
Ancillary Study II	50
Appendix II	52
Appendix III	52
Appendix IV	52

Précis

- Up to 140 patients with chronic hepatitis C, genotypes 1-4, who were never treated or previously treated but failed a course of therapy with any interferon and ribavirin combination regimen will be eligible to be enrolled into this pilot study to evaluate the combination of sofosbuvir and GS-5816 as a fixed dose tablet to improve response to antiviral therapy. To enrich the study population with subjects with a greater likelihood of virological relapse after stopping therapy, we plan to enroll a minimum of 60% treatment-experienced subjects and 50% with cirrhosis. These two drugs inhibit key enzymes that are necessary for viral replication. Sofosbuvir, an NS5B polymerase inhibitor is already approved for use in combination with interferon and ribavirin for the treatment of HCV genotype 1 infection. GS-5816 is an NS5A replication complex inhibitor with potent activity against most strains of hepatitis C virus. Combining these two agents into a single pill should improve patient compliance and improve tolerability because interferon and ribavirin will not be part of the regimen. After medical evaluation and liver biopsy, patients will receive combination therapy with sofosbuvir and GS-5816 one pill a day for 12 weeks. The baseline liver biopsy is necessary to assess the amount of liver damage caused by the HCV and to measure expression of genes associated with clearance of HCV. Blood samples will be collected to monitor safety and response to therapy and for research purposes. HCV RNA levels will be monitored frequently for the initial 4 weeks and then at monthly intervals for the remaining 8 weeks of antiviral therapy. All subjects will undergo a second liver biopsy, 4 weeks after starting therapy. The second biopsy is being performed for research purposes so investigators can determine specifically which liver genes are associated with failure of therapy (and response to therapy). Subjects who refuse the second liver biopsy will continue to receive SOF/GS-5816 treatment for the planned 12 week duration. Patients in whom serum HCV RNA is \geq lower limit of quantification (LLOQ) after 2 consecutive HCV RNA $<$ LLOQ or who have a confirmed $> 1 \log_{10}$ increase from nadir will discontinue therapy (because continuing therapy is considered futile i.e. it is unlikely to work). The major endpoints will be changes in interferon stimulated gene and protein expression in the liver and changes in HCV RNA levels in liver and serum between baseline and 4 weeks and rates of sustained virologic response at post-treatment week 12. Secondary endpoints will be safety and sustained virologic response at post-treatment week 24 weeks. (Word count =370)

A. Background: Chronic Hepatitis C and Current Therapy:

Chronic hepatitis C (CHC) is a global health problem. The World Health Organization (WHO) estimates that up to 3% of the world's population is infected with HCV amounting to an estimated 170 million to 200 million chronic HCV infections worldwide.^{1, 2} The prevalence varies with geographic region, with the highest regions being Southeast Asia and Africa.³⁻⁵ The Centers for Disease Control estimates that there are approximately 3.2 million people chronically infected with hepatitis C virus (HCV) in the United States.⁶ CHC is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma. A recent analysis suggested that the number of cases of cirrhosis in persons who were infected in the 70s and 80s will not peak until 2020;⁷ without therapy, a significant proportion of these persons will develop complications of their disease. In 2007 alone, it is estimated that over 15,000 people in the United States died from HCV-related complications. HCV now surpasses human immunodeficiency virus (HIV) as a cause of death in the United States.⁸

The recommended therapy for chronic hepatitis C genotype 1 infection used to be a 48-week course of the combination of peginterferon (1.5 µg/kg [PegIntron] or 180 µg [Pegasys] once weekly) and ribavirin (600 to 1200 mg daily based upon body weight).⁹ Recommendations changed in 2011 when two new agents, boceprevir and telaprevir, were approved for use in CHC genotype 1 infection.^{10, 11} Both agents are NS3/NS4A protease inhibitors specific for HCV genotype 1 and must be used in combination with peginterferon and ribavirin. Both agents were shown to significantly increase the SVR rate compared to standard peginterferon and ribavirin therapy. Thus, in HCV genotype 1 patients, the SVR rates were increased from 40-45% with standard combination therapy to 69-75% with triple therapy.^{11, 12} Boceprevir and telaprevir were associated with additional morbidity and mortality, above and beyond that which results from peginterferon and ribavirin. Skin rash, anemia and ano-rectal discomfort were dose-limiting side effects of telaprevir,¹¹ whereas anemia and dysgeusia were the major additional adverse events associated with boceprevir.¹² Response rates varied greatly by virologic, host and disease characteristics, such as viral level (higher rates with lower baseline HCV RNA concentrations), race (lower in African Americans, higher in Asians), and disease severity (lower in patients with cirrhosis or advanced fibrosis).^{11, 12} Treatment recommendations for HCV genotype 1 were updated again in 2013, with the approval of another once daily HCV protease inhibitor simiprevir and a NS5B polymerase inhibitor, sofosbuvir, both used on combination with peginterferon and ribavirin for 24 and 12 weeks, respectively.¹³⁻¹⁵ These new regimens represented substantial improvement over existing regimens in that they were of shorter duration, much simpler to administer, associated with less side effects and more efficacious.¹³⁻¹⁵

For patients with genotype 2 and 3, the recommended therapy is a 12 to 24 week course of the combination of sofosbuvir and weight-based ribavirin.¹⁶ The optimal regimen for patients with genotypes 4, 5 and 6 is yet to be defined.¹⁶

The development of an IFN-free regimen for the treatment of chronic HCV infection has the potential to have a major impact on the global incidence, prevalence and burden of disease due to HCV infections. New treatment options are especially crucial in patient populations for whom treatment with peginterferon is not possible, undesired, or without

sufficient efficacy. These interferon free treatments hold the promise for simpler regimens with short duration of therapy, minimal side effects and high efficacy. One such regimen is the combination of sofosbuvir and GS-5816. A 12 week course of this regimen is associated with sustained viral eradication in $\geq 90\%$ of subjects with genotypes 1-6 infection.¹⁷ Other interferon free regimens are being developed and evaluated but none have such a broad activity against all the major HCV genotypes.

Results of the phase 3 trial evaluating the efficacy of sofosbuvir and ribavirin for genotypes 2 and 3 chronic HCV infection noted a marked difference in response rates between genotypes 2 and 3-genotype 2 subjects had an SVR rate of 97% compared to 56% for genotype 3 subjects.¹³ The primary reason for treatment failure among HCV genotype 3 subjects was virological relapse. The cause for this high relapse rate is unknown. In addition, the usual cause for failure of interferon-free regimens in other viral genotypes, although much lower, is predominantly virological relapse. We hypothesize that a strong innate immune response in addition to the potency of the antiviral agents are necessary to achieve an SVR during therapy with direct acting antiviral agents and that the innate immune response is reduced among genotype 3 subjects compared to genotype 2 subjects and among relapsers of other genotypes in general.

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate diverse biological processes primarily through control of expression of cellular messenger RNAs (mRNAs).¹⁸ MiRNAs target and regulate essentially all biological processes and cell types, including those in the liver. miRNAs have been detected in serum and plasma, called circulating miRNAs and distinct patterns have been described among the healthy and diseased state.^{19, 20} Thus, they may have value as potential biomarkers. For example, circulating mir-122 and mir-155 were identified as inflammatory markers in different forms of liver injury.²¹⁻²³ Furthermore, mir-122 has been shown to be important for HCV replication.^{24, 25} Indeed, an inhibitor of mir-122 is in clinical trials as a therapeutic approach to treating chronic HCV infection.²⁶ However, little is known whether miRNAs may play a role in eradication of HCV or could be used as an early predictor of response to therapy.

The primary focus of this proposal will be to evaluate the clinical safety and efficacy of sofosbuvir and GS-5816 among HCV genotypes 1-4 (since both sofosbuvir and GS-5816 have pangenotypic activity) and to understand the mechanisms of virological relapse; specifically the role of the antiviral innate immune system (induction of antiviral interferon-stimulated genes and natural killer cells) and miRNA in clearance of HCV, particularly between HCV genotypes 2 and 3. We propose to study both the role of endogenous ISG gene expression and miRNA and to correlate it with HCV clearance. Untreated and previously treated subjects, with HCV genotypes 1-4 who have failed to achieve SVR will be assessed for IL28b genotype, ISG gene expression before and during therapy, viral kinetic response to treatment and circulating and intrahepatic miRNA levels during treatment with an interferon-free regimen consisting of sofosbuvir and GS-5816. The study population will be enriched with treatment-experienced subjects with cirrhosis for two reasons: first this is a patient population of highest urgency to be treated because of an increased risk of progressive liver disease and complications from HCV and second to increase the chance of failure with sofosbuvir and GS-5816 to allow for accrual of relapse patients.

B. Hypothesis and Aims:

B.1 Hypothesis:

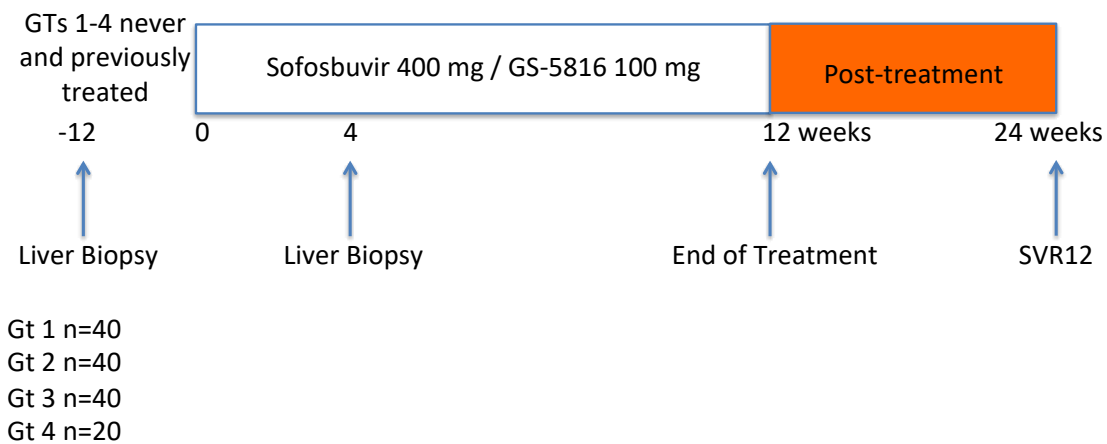
- Phase 1 viral kinetics will vary among different HCV genotypes and will be predictive of relapse following 12 weeks of therapy.
- Relapse is related to:
 - i) differences in early viral kinetics
 - ii) differences in innate immune response
 - iii) different intrahepatic and circulating miRNA profiles
 - iv) presence of hepatic steatosis and insulin resistance

B.2 Aims:

- 1) Assess safety and efficacy of sofosbuvir and GS-5816 among subjects with HCV genotypes 1-4
- 2) Assess innate immune response, miRNA and early viral kinetics in patients with chronic hepatitis C infected with HCV genotypes 1-4.
- 3) Correlate early viral kinetics, SVR and relapse with degree of hepatic steatosis and presence of insulin resistance.

C. Trial design

Safety And Efficacy OF Sofosbuvir And GS-5816 Fixed Dose Combination In HCV Genotypes 1-4



D. Summary Of Investigational Agents

D.1.Sofosbuvir (SOF) (formerly GS-7977)

D.2. GS-5816

D.3. SOF/GS-5816 Fixed Dose Combination (FDC)

Please refer to the Investigator's Brochures (IB) for information on SOF and GS-5816 including:

- In Vitro Anti-HCV Activity
- Nonclinical Pharmacokinetics and In Vitro Metabolism
- Nonclinical Pharmacology and Toxicology

Please refer to Appendix II for Clinical Experience with SOF and GS-5816.

E. Protocol

Up to 140 patients with chronic hepatitis C, genotypes 1-4, who were never treated or previously treated but failed a course of therapy with any interferon and ribavirin combination regimen will be eligible to be enrolled into this pilot study to evaluate the combination of sofosbuvir and GS-5816 as a fixed dose tablet to improve response to antiviral therapy. This regimen was approved by the Food and Drug Administration in May 2016 for the treatment of adults with genotype 1-6 chronic hepatitis C virus infection. So it is possible to obtain the same treatment outside of this protocol and participation in this study is now an alternative to standard clinical care. To enrich the study population with subjects with a greater likelihood of virological relapse after stopping therapy, we plan to enroll a minimum of 60% treatment-experienced subjects and 50% with cirrhosis. These two drugs inhibit key enzymes that are necessary for viral replication. Sofosbuvir, an NS5B polymerase inhibitor is already approved for use in combination with interferon and ribavirin for the treatment of HCV genotype 1 infection. GS-5816 is an NS5A replication complex inhibitor with potent activity against most strains of hepatitis C virus. Combining these two agents into a single pill should improve patient compliance and improve tolerability because interferon and ribavirin will not be part of the regimen. After medical evaluation and liver biopsy, patients will receive combination therapy with sofosbuvir and GS-5816 one pill a day for 12 weeks. The baseline liver biopsy is necessary to assess the amount of liver damage caused by the HCV and to measure expression of genes associated with clearance of HCV. Blood samples will be collected to monitor safety and response to therapy and for research

purposes. HCV RNA levels will be monitored frequently for the initial 4 weeks and then at monthly intervals for the remaining 8 weeks of antiviral therapy. All subjects will undergo a second liver biopsy, 4 weeks after starting therapy. The second biopsy is being performed for research purposes so investigators can determine specifically which liver genes are associated with failure of therapy (and response to therapy). Subjects who refuse the second liver biopsy will continue to receive SOF/GS-5816 treatment for the planned 12 week duration. Patients in whom serum HCV RNA is \geq lower limit of quantification (LLOQ) after 2 consecutive HCV RNA $<$ LLOQ or who have a confirmed $> 1 \log_{10}$ increase from nadir will discontinue therapy (because continuing therapy is considered futile i.e. it is unlikely to work).

The major endpoints will be changes in interferon stimulated gene and protein expression in the liver and changes in HCV RNA levels in liver and serum between baseline and 4 weeks and rates of sustained virologic response at post-treatment week 12. Secondary endpoints will be safety and sustained virologic response at post-treatment week 24 weeks.

F. Study Population

140 subjects will be enrolled in this study. (40 genotype 1, 40 genotype 2, 40 genotype 3 and 20 genotype 4-treatment naïve or experienced).

F.1. Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study.

- 1) Willing and able to provide written informed consent.
- 2) Male or female, age ≥ 18 years.
- 3) Body mass index (BMI) ≥ 18 kg/m².
- 4) HCV RNA $\geq 10^4$ IU/mL at Screening
- 5) HCV genotypes 1a, 1b, 2, 3 or 4 at screening
- 6) Confirmation of chronic HCV infection documented by either:
 - a) A positive anti-HCV antibody test or positive HCV RNA or positive HCV genotyping test at least 6 months prior to the Baseline/Day 1 visit, or
 - b) A liver biopsy performed within 12 weeks prior to the Baseline/Day 1 visit with evidence of chronic HCV infection. A prior biopsy would be acceptable if performed with 12 weeks AND liver tissue stored in RNALater was available.
- 7) Screening ECG without clinically significant abnormalities.

- 8) Subjects must have the following laboratory parameters at screening:
- a) ALT $\leq 10 \times$ the upper limit of normal (ULN)
 - b) AST $\leq 10 \times$ ULN
 - c) Direct bilirubin ≤ 1.5 ULN
 - d) Platelets $> 70,000$
 - e) HbA_{1c} $\leq 8.5\%$
 - f) eGFR ≥ 60 mL /min, as calculated by the CKD-EPI equation.
 - g) Hemoglobin ≥ 10 g/dL.
 - h) Albumin ≥ 3 g/dL
 - i) INR $\leq 1.5 \times$ ULN unless subject has known hemophilia or is stable on an anticoagulant regimen affecting INR.
- 9) A female subject is eligible to enter the study if it is confirmed that she is:
- a) Not pregnant or nursing
 - b) Of non-childbearing potential (i.e., women who have had a hysterectomy, have both ovaries removed or medically documented ovarian failure, or are postmenopausal - women > 50 years of age with cessation (for ≥ 12 months) of previously occurring menses),
- OR
- c) Of childbearing potential (i.e., women who have not had a hysterectomy, have not had both ovaries removed, and have not had medically documented ovarian failure). Women ≤ 50 years of age with amenorrhea will be considered to be of childbearing potential. These women must have a negative serum pregnancy test at Screening and a negative urine pregnancy test on the Baseline/Day 1 visit prior to randomization. They must also agree to one of the following from 3 weeks prior to Baseline/Day 1 until 90 days after last dose of study drug:—
 - i) Complete abstinence from intercourse. Periodic abstinence from intercourse (e.g., calendar, ovulation, symptothermal, post-ovulation methods) is not permitted.
 - ii) Consistent and correct use of 1 of the following methods of birth control listed below in addition to a male partner who correctly uses a condom from the date of Screening until 90 days after last dose of study drug:
 - (1) intrauterine device (IUD) with a failure rate of $< 1\%$ per year
 - (2) female barrier method: cervical cap or diaphragm with spermicidal agent

- (3) tubal sterilization
- (4) vasectomy in male partner
- (5) hormone-containing contraceptive:
 - 1. implants of levonorgestrel
 - 2. injectable progesterone
 - 3. oral contraceptives (either combined or progesterone only)
 - 4. contraceptive vaginal ring
 - 5. transdermal contraceptive patch

- 10) All male study participants must agree to consistently and correctly use a condom, while their female partner agrees to use 1 of the methods of birth control listed above, from the date of Screening until 90 days after last dose of study drug.
- 11) Male subjects must agree to refrain from sperm donation from 90 days after their last dose of study drug.
- 12) Subject must be of generally good health, with the exception of chronic HCV infection, as determined by the Investigator.
- 13) Subject must be able to comply with the dosing instructions for study drug administration and able to complete the study schedule of assessments, including all required post-treatment visits.

F.2. Exclusion Criteria

Subjects who meet *any* of the following exclusion criteria will 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) Difficulty with blood collection and/or poor venous access for the purposes of phlebotomy.

- 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) Psychiatric hospitalization, suicide attempt, and/or a period of disability as a result of their psychiatric illness within the last 5 years. Subjects with psychiatric illness (without the prior mentioned conditions) that is well-controlled on a stable treatment regimen for at least 12 months prior to randomization or has not required medication in the last 12 months may be included.
 - h) Malignancy within 5 years prior to screening, with the exception of specific cancers that are entirely cured by surgical resection (basal cell skin cancer, etc). Subjects under evaluation for possible malignancy are not eligible.
 - i) Significant drug allergy (such as anaphylaxis or hepatotoxicity).
- 2) Any prior treatment with a direct acting antiviral agent (protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors/non-nucleoside polymerase inhibitors.)
 - 3) Pregnant or nursing female or male with pregnant female partner.
 - 4) Chronic liver disease of a non-HCV etiology (e.g., hemochromatosis, Wilson's disease, alfa-1 antitrypsin deficiency, cholangitis).
 - 5) Infection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV).
 - 6) Clinically-relevant drug abuse within 12 months of screening. A positive drug screen will exclude subjects unless it can be explained by a prescribed medication; the diagnosis and prescription must be approved by the investigator.
 - 7) Use of any prohibited concomitant medications as described in Section G.3 and Table 1 within 21 days of the Baseline/Day 1 visit; this washout period does not apply to PPIs, which can be taken up to 7 days before baseline Day 1.
 - 8) Use of antiviral medications within the last 30 days.
 - 9) Chronic use of systemically administered immunosuppressive agents (e.g., prednisone equivalent > 10 mg/day).
 - 10) Known hypersensitivity to GS-5816, SOF, or formulation excipients.

G. Treatments

G.1 Description and Handling of SOF/GS-5816 FDC

G.1.1 Formulation

Sofosbuvir/GS-5816 FDC tablets, 400 mg/100 mg, are available as pink, diamond-shaped, film-coated tablets debossed with “GSI” on one side and “7916” on the other side. In addition to the active ingredients, SOF/GS-5816 FDC tablets also contain copovidone, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyvinyl alcohol, titanium dioxide, macrogol, talc, and iron oxide red.

G.1.2 Packaging and Labeling

Sofosbuvir/GS-5816 FDC and placebo-to-match tablets are packaged in white, high-density polyethylene bottles. Each bottle contains 28 tablets and polyester packing material. Each bottle is enclosed with a white, continuous thread, child-resistant screw cap with an induction-sealed, aluminum-faced liner.

G.1.3 Storage and Handling

Sofosbuvir/GS-5816 FDC bottles should be stored at controlled room temperature until required for administration. Controlled room temperature is defined as 25°C (77°F); excursions are permitted between 15°C and 30°C (59°F to 86°F).

Sofosbuvir/GS-5816 FDC products should be stored in a securely locked area, accessible only to authorized site personnel. To ensure the stability of the study drug and to ensure proper product identification, the drug product should not be stored in a container other than the container in which they are supplied. Consideration should be given to handling, preparation, and disposal through measures that minimize drug contact with the body. Appropriate precautions should be followed to avoid direct eye contact or exposure through inhalation when handling SOF.

G.1.4 Stability

It is the policy of Gilead Sciences to monitor the stability of each formulation of clinical drug product until the time when the shelf life or a reevaluation period is established for the drug product. Stability studies evaluate the labeled storage conditions and accelerated conditions, if appropriate. The frequency of stability testing is every 3 months for the first year, every 6 months for the second year, and annually thereafter for a minimum of the clinical study duration. Studies conducted under accelerated conditions serve to identify any stability trend before its occurrence under the labeled storage conditions.

Through the combined approach of accelerated-condition studies and long-term stability monitoring with frequent testing, the purported identity, strength, and quality will be assured for all lots of drug product used in clinical studies.

G.1.5 Dosage and Administration of SOF/GS-5816 FDC

Sofosbuvir/GS-5816 FDC tablet is to be administered once daily with or without food. Each subject must be given instructions to maintain approximately the same daily dosing interval between study drug doses.

For missed dose(s) of study drug, subjects should be instructed to take the missed dose(s) of study drug as soon as possible during the same day. Subjects should be cautioned never to double the next dose with a missed dose of study drug under any circumstances.

G.2. Study Drug Adherence and Drug Accountability

Compliance will be documented through the use of pill counts and patient diaries. Subjects will be instructed to bring unused study medication containers to each 4-week visit as well as any empty bottles. The dates and number of tablets dispensed and returned will be recorded by the research nurse. Open bottles of SOF/GS-5816 are collected every four weeks and new bottles will be dispensed by the Clinical Center Pharmacy. Subjects will be instructed to record dosing in a dosing diary, which will be reviewed at each visit, in combination with drug accountability to confirm treatment compliance.

G.3. Concomitant Medications

The following medications are prohibited during the screening period and for a minimum of 21 days prior to the Baseline/Day 1 visit through the end of treatment:

- Hematologic stimulating agents (eg, erythropoiesis-stimulating agents (ESAs); granulocyte colony stimulating factor (GCSF); thrombopoietin (TPO) mimetics)
- Chronic use of systemic immunosuppressants including, but not limited to, corticosteroids (prednisone equivalent of > 10 mg/day for > 2 weeks), azathioprine, or monoclonal antibodies (eg, infliximab)
- Investigational agents or devices for any indication

Concomitant use of amiodarone is prohibited during the screening period and for a minimum of 60 days prior to the Baseline/Day 1 visit through the end of treatment.

Concomitant use of certain medications or herbal/natural supplements (inhibitors or inducers of drug transporters ie, P-gp) with study drug(s) may result in PK interactions resulting in increases or decreases in exposure of study drug(s) and **are prohibited from 21 days prior to Baseline/Day 1 through the end of treatment. Examples of representative medications are listed below:**

Table 5-1. List of Disallowed Medications

Drug Class	Agents Disallowed	Use with Caution
Acid Reducing Agents ^a	Proton-Pump Inhibitors	H2-Receptor Antagonists, Antacids
Anticonvulsants ^b	Phenobarbital, Phenytoin, Carbamazepine, Oxcarbazepine	
Antimycobacterials ^b	Rifabutin, Rifapentine, Rifampin	
Cardiac Medications ^c		Diltiazem, Verapamil, Dronedaron, Amiodarone, Ranolazine, Bosentan, Olmesartan, Valsartan, Quinidine, Digoxin ^d
Herbal/Natural Supplements ^b	St. John's Wort, Echinacea, Milk thistle (i.e. silymarin), Chinese herb sho-saikoto (or Xiao-Shai-Hu-Tang)	
HMG-CoA Reductase Inhibitors ^e		Rosuvastatin (≤ 10 mg/day), Atorvastatin, Simvastatin, Pravastatin, Pitavastatin, Fluvastatin, Lovastatin
Other	Modafinil ^b , Sulfasalazine ^c , Methotrexate ^c	

- a The 21 day washout period does not apply to Proton-Pump Inhibitors, which can be taken up to 7 days before baseline Day 1. H2-receptor antagonists must not exceed a dose of 20 mg famotidine or equivalent and can be taken simultaneously with SOF/GS-5816 and/or staggered by 12 hours. Antacids that directly neutralize stomach acid (i.e. Tums, Maalox) may not be taken within 4 hours (before or after) of SOF/GS-5816 administration.
- b May result in a decrease in the concentration of study drugs.
- c May result in an increase in the concentration of study drugs and/or concomitant medications
- d Monitor for signs and symptoms of digoxin toxicity.
- e Use with SOF/GS-5816 may result in an increase in the concentration of HMG-CoA Reductase Inhibitors. Monitor for signs and symptoms of muscle weakness or myopathy, including rhabdomyolysis.

Medications for disease conditions **excluded** from the protocol (eg, HIV-1 infection, active cancer, transplantation) are not listed under this Concomitant Medication section and are disallowed in the study.

H. Study Procedures

There will be a total of 13 study visits comprising of a screening visit (which may be completed with the admission for liver biopsy/initiation of therapy visit), on-treatment weeks 1, 2, and 3, admission for repeat biopsy/week 4 visit, outpatient week 8 and 12 visits and post-treatment visits weeks 2, 4, 8, 12, and 24.

Information on the specific clinical assessments and laboratory parameters to be monitored are provided below.

H.1 Consent Process

Potential study subjects will be informed of study rationale, design, participation burden, risks, benefits and side effects of therapy during routine LDB clinic visits and will have an opportunity to ask questions about the proposed study. They will be provided a copy of the study consent form to take home and review in more detail. If a subject indicates a desire to participate in the research study they will be asked to sign consent after which, he/she will have all screening laboratories, radiologic imaging and if necessary, liver

biopsy, performed through the current protocol. The initial liver biopsy for participation in this study will be medically indicated and for research purposes. However, the second liver biopsy will be only for research purposes. Patients will be made aware of this.. Subjects who refuse the second liver biopsy will continue to receive SOF/GS-5816 treatment for the planned 12 week duration. The final study sample size accounts for a drop out rate of up to 20%. Patients will only be replaced if the dropout rate exceeds this proportion. Subjects who are eligible to participate in the ancillary study and agree to undergo lymphapheresis will be consented via a separate consent under this protocol.”

H.1.2 Short Form Consent

We do not plan to enroll non-English speaking subjects; however they are not excluded from participation either. Should a non-English speaking subject be enrolled, IRB approval will be obtained to use the short form process in the absence of a fully translated consent document. Requests for IRB approval will be obtained prior to implementing the short form consent process. The short form consent process will be in compliance with SOP 12.9.1 of 45 CFR 46.117(b)(2).

H.2 Investigator Responsibilities

The PI or physician associate investigator following the patient is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The PI and AIs are also responsible for ensuring that signed consents and documentation of the consent process are both placed in the subject’s permanent CC and/or electronic medical record (CRIS). The Investigators responsible for obtaining informed consent are as follows:

- Dr. Marc Ghany
- Dr. Jake Liang
- Dr. Edward Doo
- Dr. Theo Heller
- Dr. Yaron Rotman
- Dr. Christopher Koh
- Dr. Devika Kapuria
- Dr. Ahmad Alawad
- Dr. Gil Ben Yakov
- Dr. Walter Lai

These Investigators will also be responsible for assessment of AEs, side effects, and patient response to treatment. The RN associate investigators will be responsible for scheduling patients, completing study drug logs, assisting patients with completing questionnaires, and medication reconciliation.

H.3. Pre-treatment Phase: (Screening)

Patients will be seen by the Liver Diseases Branch of NIDDK, in the outpatient clinic of the Clinical Center, NIH, under protocol 91-DK-0214, where they will be assessed for enrollment based upon history of never receiving therapy, or failure of a prior adequate course of interferon-based and ribavirin and HCV genotype. An adequate course of therapy is defined as a minimum of 24 weeks of interferon and ribavirin for relapsers and partial responders and 12 weeks for null responders. Patients who qualify will be approached regarding enrollment and the risks and benefits discussed in detail. Patients who qualify and agree will be asked to sign the consent form. Patients will undergo screening labs as outpatients, and then be scheduled for inpatient admission within 12 weeks to complete the screening assessment, undergo liver biopsy and start therapy. Patients may have the option to defer initiation of therapy if they so wish but they should begin therapy within 12 weeks of the screening assessment. If additional testing is required, then patients will have up to 20 weeks from the Screening visit to start study treatment. A prior biopsy would be acceptable in lieu of the baseline biopsy ONLY if performed with 12 weeks of starting therapy AND liver tissue stored in RNA Later was available.

H.3.1 Outpatient Screening Visit:

- (1) Obtain signed informed consent
- (2) History and physical examination
- (3) Symptom questionnaire
- (4) Fatigue questionnaire (Promise 7)
- (5) Visual analogue scale
- (6) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count), HgbA1C, and HCV RNA levels. Testing will also be done for anti-HAV, HBsAg, anti-HBs, anti-HBc, anti-HCV, HCV genotype, anti-HIV-1.
- (7) Extended blood panel: alpha fetoprotein, gGT, immunoglobulins, rheumatoid factor, ANA, SMA, lipid panel, thyroid panel, INR, reticulocyte count, ESR.
- (8) Serum pregnancy test for females of childbearing potential
- (9) Research blood for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots). The stored serum will be used to measure selected cytokines, interferon-stimulated gene products [including IP10] and HCV RNA repeat testing or sequencing as needed and for future research.
- (10) Research blood for ancillary study (40mL of blood in heparin)
- (11) Urine tests: routine urinalysis

H.3.2 Ancillary Study procedure

Genotype 1 HLA-2A+ patients who agree will undergo double-pass lymphopheresis (preferred) or undergo blood draw of 100 ml of blood for ancillary study I.

H.4 Completion of Screening / Admission for Pre-Treatment Liver Biopsy / Start of Treatment:

Patients who are not excluded based on outpatient screening will be admitted to the Clinical Center as inpatients to complete screening tests (ultrasound, EKG), undergo the pre-treatment liver biopsy, to start treatment, and for timed blood draws. Patients will have the option to divide this visit into two visits, completing the screening assessments and liver biopsy over three days and then being re-admitted at a later date for four days to begin treatment. Patients who elect to divide this visit into two should initiate therapy within 12 weeks after the screening assessment.

- 1) 12-lead EKG
- 2) Transient elastography (Fibroscan)
- 3) Abdominal ultrasound
- 4) Brief clinical evaluation
- 5) Review patient's eligibility for study
- 6) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, plasma lipids and lipoproteins, serum insulin, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 7) Liver biopsy cut into four sections, one (at least 1.5 cm) placed in formalin for routine light microscopy and the other 3 (at least 0.3 cm) placed in RNAlater and flash frozen in liquid nitrogen for RNA and protein analyses and flow cytometry. Patients with a transient elastography score ≥ 13 will undergo a transjugular liver biopsy with concomitant measurement of wedged and free hepatic vein pressure to estimate the hepatic portal venous gradient
- 8) Symptom questionnaire
- 9) Visual analogue scale
- 10) Fatigue questionnaire (Promise 7)
- 11) Review concomitant medications for possible drug interactions
- 12) Females of child bearing potential: Urine pregnancy test
- 13) Research Blood for Ancillary Study I (40mL blood in heparin)
- 14) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 15) Start Sofosbuvir/GS-5816
- 16) HCV RNA levels prior to starting therapy, then at 6, 12 and 18 hours

Days 1, 2 and 3, completed during inpatient admission:

- 1) Assessment for side effects/adverse events
- 2) HCV RNA levels at 24, 48 and 72 hours.
- 3) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 4) Blood sample for plasma lipids and lipoproteins
- 5) Discharge from hospital after blood draw on day 3

If a patient chooses to separate the biopsy and start of treatment admission by more than one week, an additional 40ml blood for Ancillary Study I will be collected at biopsy as well as start of treatment.

H.5 Monitoring

Patients will be monitored closely for the first four weeks of treatment, and then monthly after that until completing treatment at week 12.

H.5.1 Weeks 1, 2 & 3, (\pm 3 days):

Outpatient visit

- 1) Symptom questionnaire
- 2) Visual analogue scale
- 3) Fatigue questionnaire (Promise 7) (week 2 only)
- 4) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 5) HCV RNA levels weeks 1, 2, 3, and 4
- 6) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 7) Week 1 only: plasma lipids and lipoproteins, Serum Insulin
- 8) Week 2 only: Research blood for ancillary study I (40mL blood in heparin)

H.5.2. Week 4: (\pm 5 days)

Admission to the clinical center will occur within the fourth week after starting treatment.

- 1) Admission to hospital
- 2) Symptom questionnaire
- 3) Fatigue questionnaire (Promise 7)
- 4) Visual analogue scale
- 5) Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, plasma lipids and lipoproteins, serum insulin, Hemoglobin A1c, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 6) HCV RNA level
- 7) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 8) Research blood for ancillary study I (40mL of blood in heparin)
- 9) Females of child bearing potential: Pregnancy test

- 10) Liver biopsy cut into four sections, one (at least 1.0 cm) placed in formalin for routine light microscopy and three sections (at least 0.3 cm) placed in RNAlater and flash frozen in liquid nitrogen for RNA and protein analyses.

H.5.3 Week 8 (\pm 3 days):

After 4 weeks patients will continue to receive sofosbuvir and GS-5816 for a total of 12 weeks. For participants having side effects related to treatment or adherence issues, more frequent visits can be undertaken at the discretion of the study physician. Patients in whom HCV RNA is \geq LLOQ after 2 consecutive HCV RNA $<$ LLOQ or who have a confirmed $>$ 1 log₁₀ increase from nadir will discontinue therapy (stopping rule for futility).

Each outpatient visit will include assessment of compliance, adverse events reporting, vital signs, weight, symptoms evaluation, record of concomitant medications, drug dispensing

- 1) Symptom questionnaire
- 2) Visual analogue scale
- 3) Fatigue questionnaire (Promise 7)
- 4) Blood will be drawn for Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 5) HCV RNA level
- 6) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 7) Females of child bearing potential: Pregnancy test

H.5.4 End of Treatment (Week 12 \pm 3 days))

At week 12, all subjects will discontinue therapy and undergo the following evaluation:

- 1) Physical examination
- 2) Symptom questionnaire
- 3) Fatigue questionnaire (Promise 7)
- 4) Visual analogue scale
- 5) Assessment of adverse events, concomitant medications and adherence
- 6) Blood will be drawn for Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, plasma lipids and lipoproteins, serum insulin, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 7) Research blood for ancillary study I (40mL blood in heparin for genotype 2, 3, 4 and 1 patients who are not HLA-A2+)

Urine tests: routine urinalysis and creatinine, phosphate and beta-2-microglobulin

- 8) HCV RNA level.
- 9) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 10) Females of child bearing potential: Pregnancy test

H.5.5 Ancillary Study Procedure

For genotype 1 patients who are HLA-A2+, lymphapheresis or 100mL blood in heparin will be performed within 24 weeks of stopping treatment.

H.6 Post-treatment weeks 2, 4, 8 and 12 (\pm 5 days)

Patients who complete 12 weeks of therapy will be seen at weeks 2, 4, 8, 12 and 24 after therapy is stopped. Two criteria for a sustained virologic response will be used (HCV RNA negative at 12 [SVR 12] and 24 weeks [SVR 24] after stopping therapy).

- 1) Limited clinical evaluation
- 2) Symptom questionnaire
- 3) Visual analogue scale
- 4) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 5) HCV RNA level
- 6) Research blood for ancillary study I (40mL blood in heparin for genotype 2, 3 and 4 patients)
- 7) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 8) Females of child bearing potential: Urine pregnancy test (week 4, 8 and 12)
- 9) Weeks 4 and 12 only: plasma lipids and lipoproteins, Serum Insulin
- 10) Week 12 only: Hemoglobin A1c

H.7 Final study visit week 24 post-treatment (\pm 5 days)

- 1) Limited clinical evaluation
- 2) Symptom questionnaire
- 3) Fatigue questionnaire (Promise 7)
- 4) Visual analogue scale
- 5) Routine blood panel: Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), lipase, plasma lipids and lipoproteins, serum insulin, Hemoglobin A1c, CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).

- 6) Extended blood panel: alpha fetoprotein, gGT, immunoglobulins, rheumatoid factor, ANA, SMA, lipid panel, INR, reticulocyte count, ESR.
- 7) Research blood for ancillary study I (40mL blood in heparin)
- 8) HCV RNA level
- 9) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 10) Transient elastography

For patients who fail treatment, resistance testing will be carried out to post-treatment week 48

I. Discontinuation of Subjects from Treatment

I.1 Virologic Response-Based Treatment Stopping Criteria:

The following on-treatment virologic response-based treatment stopping criteria will be utilized:

- Confirmed HCV RNA \geq LLOQ after 2 consecutive HCV RNA $<$ LLOQ
- Confirmed $> 1 \log_{10}$ increase from nadir
- Confirmation should be performed as soon as possible and must occur no later than 2 weeks after an initial observation indicating virologic failure during the on-treatment phase.

I.2 Other Stopping Criteria:

- Unacceptable toxicity, as defined in the protocol, or toxicity that, in the judgment of the investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
- Pregnancy of female subject
- Significant protocol violation that adversely affects the health care of the subject(s) or compromises the interpretation or integrity of the research
- Subject request to discontinue for any reason; it is important to determine whether the withdrawal of consent is primarily due to an AE, lack of efficacy or other reason
- Discontinuation of the study at the request of Gilead, regulatory agency or an Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

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

- Elevation of ALT and/or AST $> 5x$ Baseline/Day 1 or nadir, confirmed by immediate repeat testing

- Abnormal elevation of ALT > 3 x Baseline/Day 1 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 adverse event or laboratory abnormality assessed as related to SOF/GS-5816 FDC

Gilead Sciences will be notified as soon as possible, when a subject comes off treatment due to an AE.

I.3 Early Termination (ET) Visit

If a subject discontinues treatment early for any reason then the following assessments for the ET Visit will be performed:

- 1) Physical examination
- 2) Symptom questionnaire
- 3) Fatigue questionnaire (Promise 7)
- 4) Visual analogue scale
- 5) Assessment of adverse events, concomitant medications and adherence
- 6) Blood will be drawn for Chemistry 20 (sodium, chloride, potassium, bicarbonate, calcium, phosphate, magnesium, glucose, blood urine nitrogen, creatinine, uric acid, ALT, AST, LDH, CPK, Alkaline Phosphatase, total and direct bilirubin, albumin, total protein), CBC (hematocrit, hemoglobin, white blood cell count and differential, platelet count).
- 7) Research blood for ancillary study I
- 8) HCV RNA level.
- 9) 12 lead EKG
- 10) Blood sample for storage (5 mL of blood separated into serum and stored frozen in 0.5-1.0 mL aliquots)
- 11) Females of child bearing potential: Pregnancy test
- 12) Assess compliance with study drug dosing regimen including pill count and review of dosing diary data with subject

All subjects who terminate treatment early will complete the ET visit, and the 4, 8, 12 and 24-Week Post Treatment visits, based on their last dose date.

J. Unscheduled Visits

A subject should attend an unscheduled visit if requested by the investigator. The assessments are at the investigator's discretion, and will at a minimum collect AE and concomitant medication information.

At all unscheduled visits initiated for the purpose of confirming virologic failure a research sample will be obtained for Viral RNA Sequencing / Phenotyping.

K. Procedures

K.1 Assays for Monitoring HCV RNA levels and Genotype/Subtype determination:

The Roche COBAS AmpliPrep/ COBAS TaqMan HCV Test will be used for HCV RNA quantification and the VERSANT HCV Genotype 2.0 Assay (LiPA) will be used for HCV genotype/subtype determination. The VERSANT HCV Genotype 2.0 Assay (LiPA) targets sequence motifs from the core and 5'UTR regions of HCV.

K.2 Management of non-response

Patients in whom HCV RNA is \geq LLOQ after 2 consecutive HCV RNA $<$ LLOQ or who have a confirmed $> 1 \log_{10}$ increase from nadir will discontinue therapy (stopping rule for futility). These patients will continue to be monitored as per the post-treatment monitoring schedule. The management of non-responders to DAA regimens is unknown. These patients may be eligible for future investigational regimens at the Clinical Center or other research centers when such options become available.

K.3 Management of virological breakthrough

Patients who develop confirmed virological breakthrough (confirmed within 2 weeks of initial observation) will discontinue therapy. These patients will continue to be monitored as per the post-treatment monitoring schedule. An additional research sample will be drawn for investigation of resistance-associated variants as outlined in section Q. The management of virological resistance to sofosbuvir and GS-5816 is unknown. These patients may be eligible for future investigational regimens at the Clinical Center or other research centers when such options become available.

K.4 Medical History

Medical history including details regarding illnesses and allergies, date(s) of onset, and whether condition(s) is currently ongoing, and medication history will be collected on all subjects during screening.

K.5 Complete Physical Examination

A complete physical examination must include source documentation of general appearance, and the following body systems: Head, neck and thyroid; eyes, ears, nose, throat, mouth and tongue; chest (excluding breasts); respiratory; cardiovascular; lymph nodes, abdomen; skin, hair, nails; musculoskeletal; neurological.

K.6 Vital Signs

Vital sign collection will include measurement of resting blood pressure, pulse, respiratory rate, and temperature.

K.7 Creatinine Clearance

Renal function will be assessed by estimated glomerular filtration rate (eGFR) using the CKD-EPI equation.

The CKD-EPI equation is: $GFR = 141 \times \min(Scr/k, 1)^a \times \max(Scr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black] where SCr is serum creatinine (mg/dL), k is 0.7 for females and 0.9 for males, a is -0.329 for females and -0.411 for males, min indicates the minimum of SCr/k or 1, and max indicates the maximum of SCr/k or 1.

K.8 Body Mass Index (BMI)

BMI is calculated by the following equation.

$$BMI = \frac{\text{weight (pounds)} \times 703}{(\text{height in inches})^2} \quad \text{or} \quad \frac{\text{weight in kilograms}}{(\text{height in meters})^2}$$

K.9 12-Lead ECGs

The investigator (or qualified designee) should review the ECG traces recorded in real time for clinically significant abnormalities. On treatment ECGs, obtained for medically indicated reasons, should be compared to the subject's Baseline as part of routine safety monitoring.

K.10 Viral RNA Sequencing / Phenotyping Sample

Serum/plasma samples will be collected at each visit for viral sequence analysis. Additionally, at any unscheduled visit initiated for the purpose of confirming virologic breakthrough, a viral sequence analysis plasma sample must be collected. Unused samples may be archived.

K.11 IL28B Testing

A blood sample will be obtained at Screening for specific genetic analysis of the rs12979860 (IL28B) genetic variant. Results of the test will be made available to subjects if they wish to receive these results. Results of this test will not be used for eligibility or stratification. Samples will be analyzed retrospectively for IL-28B under our natural history protocol 91-DK-0214 which, all patients who agree to genetic testing would have signed.

K.12 Pregnancy Testing

Women of child bearing potential must have a negative serum β -HCG at screening and a negative urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of investigational product. All females of childbearing potential will have urine pregnancy testing every 4 weeks during the dosing period and for a minimum of 3 months following the last dose of sofosbuvir and GS-5816.

Specifically, urine pregnancy will be performed at treatment week 4, 8 and 12, and at post-treatment weeks 4, 8 and 12. If a positive urine pregnancy test is reported, the subject will be asked to return to the clinic for a confirmatory serum pregnancy test.

K.13 Health Related Quality of Life Surveys

Quality of life surveys included in this study are Symptom questionnaire, Visual analog scale, and Fatigue questionnaire (Promise 7) which will be completed by subjects at Day 1 (Baseline), On-treatment Weeks 2, 4, 8 and 12, Post-treatment Weeks 4, 8, 12 and 24 and Early Termination (if applicable). The subject should read the questionnaire by himself/herself and write/mark answers directly onto the questionnaire.

K.14 Nuclear Medicine Resonance Lipoprotein Profile

Plasma lipid analysis, including VLDL, LDL and HDL particle size and number will be measured by nuclear magnetic resonance (NMR) spectroscopy using the Vantera Clinical Analyzer (LipoScience Inc., Raleigh, North Carolina). VLDL-P, LDL-P, and HDL-P will be quantified based on lipoprotein particle size using the amplitudes of their distinct lipid methyl group NMR signals to calculate the different lipoproteins. Lipoprotein particles will be further subdivided into large, medium, and small particles based on the mean particle sizes as the weighted average of related subclasses.

L. Hazards and Discomforts

L.1 The risks and discomforts of frequent phlebotomy:

To document virological and biochemical response to therapy and to monitor the effects and toxicities of the combination of sofosbuvir and GS-5816, frequent blood sampling will be required. Patients will have between 15 and 18 venipunctures during the 36 weeks of the study. Each venipuncture will be for approximately 20 to 75 cc of blood. However, the total amount of blood drawn during an 8 week period will not exceed 10.5 ml/Kg (550 mls).

L.2 The risks and discomforts of HIV testing.

Patients will have blood tested for anti-HIV at entry. Mention of anti-HIV is made in the consent form which includes the exact language used in the standard consent form used for anti-HIV testing at the Clinical Center. It is important to test for HIV infection as HIV infection may affect response to therapy.

L.3 The risks and discomfort of percutaneous and transjugular liver biopsy.

Patients will undergo two liver biopsies in this protocol namely: one before therapy and a second 4 weeks later. Patients with inadequate platelet counts ($<70,000/\text{mm}^3$) or coagulation parameters (prothrombin time > 15 seconds, $\text{INR}>1.7$) will not undergo liver biopsy as a part of this protocol and will be excluded from participation in this trial. Patients already enrolled whose platelet counts fall below $70,000/\text{mm}^3$ or whose coagulation parameters exceed (prothrombin time > 15 seconds, $\text{INR}>1.7$) will not undergo the second liver biopsy but will remain on study drugs. Patients requiring liver biopsy will be admitted to the Clinical Center for two days for this procedure and other blood testing. The major side effects of liver biopsy are pain, bacteremia, puncture of another organ and bleeding. Local pain and discomfort at the liver biopsy site occurs in about 20% of persons undergoing percutaneous liver biopsy. This is transient (lasting one to twelve hours) and is usually mild, rarely requiring analgesics. Bacteremia occurs in 1-2% of persons undergoing liver biopsy. In the absence of bile duct obstruction, this is almost always self-limited and is rarely symptomatic. Significant bleeding after liver biopsy is the most serious side effect of this procedure. In the absence of a blood coagulation defect or hepatic malignancy, significant bleeding is rare, occurring in less than one in a thousand cases of liver biopsy. Death due to bleeding after liver biopsy has been reported in less than 1/10,000 cases. At the NIH Clinical Center, the Liver Diseases Branch has performed approximately 150 liver biopsies each year for the last 20 years. During this time, only three patients died as a complication of biopsy. One of these patients had cirrhosis and advanced hepatocellular carcinoma, the second had severe coagulation disorders and the third had underlying malignancy (acute lymphocytic leukemia) and a biopsy was performed for investigation of abnormal liver function tests after a bone marrow transplant. All patients bled from the liver biopsy site and died after surgical and interventional radiological attempts to stop the bleeding were unsuccessful. There is no evidence that performance of a second liver biopsy within a two week period is associated with a substantial increase in risk of complications.

L.4 The risks and hazards of sofosbuvir therapy.

The risks of sofosbuvir are discussed in detail in the drug package insert (Appendix II). The most common adverse events (incidence greater than or equal to 20%, all grades) observed with sofosbuvir in combination with ribavirin were fatigue and headache. Treatment-emergent adverse events observed in $\geq 15\%$ of subjects in clinical trials are provided in Table below.

The proportion of subjects who permanently discontinued treatment due to adverse events was 4% for subjects receiving placebo, 1% for subjects receiving sofosbuvir and ribavirin for 12 weeks, $<1\%$ for subjects receiving sofosbuvir and ribavirin for 24 weeks.

	Placebo x 12 weeks	Sofosbuvir + Ribavirin _a x 12 weeks	Sofosbuvir + Ribavirin _a x 24 weeks
--	--------------------	--	--

	N=70	N=650	N=250
Fatigue	24%	38%	30%
Headache	20%	24%	30%
Nausea	18%	22%	13%
Insomnia	4%	15%	16%
Pruritus	8%	11%	27%
Anemia	0%	10%	6%
Asthenia	3%	6%	21%
Rash	8%	8%	9%
Decreased appetite	10%	6%	6%
Chills	1%	2%	2%
Influenza like illness	3%	3%	6%
Pyrexia	0%	4%	4%
Diarrhea	6%	9%	12%
Neutropenia	0%	<1%	<1%
Myalgia	0%	6%	9%
Irritability	1%	10%	10%

- a. Subjects received weight-based ribavirin (1000 mg per day if weighing <75Kg or 1200 mg per day if weighing \geq 75Kg)

With the exception of anemia and neutropenia, the majority of events presented in the Table occurred at severity of grade 1 in sofosbuvir-containing regimens.

Less Common Adverse Reactions Reported in Clinical Trials (<1%):

The following Adverse Reactions occurred in <1% of subjects receiving sofosbuvir in a combination regimen in any one trial. These events have been included because of their seriousness or assessment of potential causal relationship.

Hematologic Effects: pancytopenia (particularly in subjects receiving concomitant pegylated interferon).

Psychiatric Disorders: severe depression (particularly in subjects with pre-existing history of psychiatric illness), including suicidal ideation and suicide.

Laboratory Abnormalities:

Changes in selected hematological parameters are described in Table 4. A side-by-side tabulation is to simplify presentation; direct comparison across trials should not be made due to differing trial designs.

Bilirubin Elevations

Total bilirubin elevation of more than 2.5xULN was observed in none of the subjects in the sofosbuvir + peginterferon alfa + ribavirin 12 weeks group and in 1%, 3% and 3% of subjects in the peginterferon alfa + ribavirin 24 weeks, sofosbuvir + ribavirin 12 weeks and sofosbuvir + ribavirin 24 weeks groups, respectively. Bilirubin levels peaked during the first 1 to 2 weeks of treatment and subsequently decreased and returned to baseline levels by post-treatment Week 4. These bilirubin elevations were not associated with transaminase elevations.

Creatine Kinase Elevations

Creatine kinase was assessed in the FISSION and NEUTRINO trials. Isolated, asymptomatic creatine kinase elevation of greater than or equal to 10xULN was observed in <1%, 1% and 2% of subjects in the peginterferon alfa + ribavirin 24 weeks, sofosbuvir + peginterferon alfa + ribavirin 12 weeks and sofosbuvir + ribavirin 12 weeks groups, respectively.

Lipase Elevations

Isolated, asymptomatic lipase elevation of greater than 3xULN was observed in <1%, 2%, 2%, and 2% of subjects in the sofosbuvir + peginterferon alfa + ribavirin 12 weeks, sofosbuvir + ribavirin 12 weeks, sofosbuvir + ribavirin 24 weeks and peginterferon alfa + ribavirin 24 weeks groups, respectively.

L.5 The risks and hazards of GS-5816 therapy

The risks of GS-5816 are discussed in detail in the Investigator's Brochure.

L.5.1 Adverse Events^[1]_{SEP}

In phase 1b and 2b clinical trials involving 377 patients with chronic hepatitis C, the AEs occurring with > 10% incidence across all treatment groups included fatigue and headache. The treatment groups administered RBV had higher incidence of AEs that have been previously observed with RBV treatment such as fatigue, headache, insomnia, and rash. Rates of AEs were similar in subjects receiving GS-5816 25 mg versus 100 mg with no discernible dose-related toxicity.

Treatment-emergent AEs occurring in at least 10% of subjects in any treatment group by preferred term are shown in Table 4-24. Additional AEs occurring in at least 10% of

subjects in any treatment group in other phase 2 studies included pruritus, upper respiratory tract infection, lethargy, back pain and vomiting.

The proportion of subjects who permanently discontinued treatment due to adverse events was 3/802 (<1%) for palpitations and dizziness (n=1), elevated ALT and gGT (n=1) and eczema infected and eye inflammation (n=1).

Table 4-24. GS-US-342-0102: Treatment-Emergent Adverse Events Occurring in at Least 10% of Subjects in Any Treatment Group by Preferred Term

	Groups 1, 3, and 5 SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 77)	Groups 2, 4, and 6 SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 77)	Groups 7 and 11 SOF 400 mg + GS-5816 25 mg 8 Weeks (N = 56)	Groups 8 and 12 SOF 400 mg + GS-5816 25 mg + RBV 8 Weeks (N = 55)	Groups 9 and 13 SOF 400 mg + GS-5816 100 mg 8 Weeks (N = 55)	Groups 10 and 14 SOF 400 mg + GS-5816 100 mg + RBV 8 Weeks (N = 57)	Total (N = 377)
Subjects with AEs	52 (67.5%)	54 (70.1%)	36 (64.3%)	45 (81.8%)	33 (60.0%)	42(73.7%)	262 (69.5%)
Fatigue	19 (24.7%)	14 (18.2%)	7 (12.5%)	17 (30.9%)	2 (3.6%)	21 (36.8%)	80 (21.2%)
Headache	16 (20.8%)	14 (18.2%)	7 (12.5%)	21 (38.2%)	9 (16.4%)	10 (17.5%)	77 (20.4%)
Nausea	10 (13.0%)	8 (10.4%)	4 (7.1%)	7 (12.7%)	7 (12.7%)	8 (14.0%)	44 (11.7%)
Diarrhoea	5 (6.5%)	7 (9.1%)	3 (5.4%)	5 (9.1%)	2 (3.6%)	6 (10.5%)	28 (7.4%)
Constipation	9 (11.7%)	6 (7.8%)	1 (1.8%)	3 (5.5%)	3 (5.5%)	1 (1.8%)	23 (6.1%)
Insomnia	3 (3.9%)	3 (3.9%)	2 (3.6%)	9 (16.4%)	1 (1.8%)	5 (8.8%)	23 (6.1%)
Nasopharyngitis	2 (2.6%)	5 (6.5%)	6 (10.7%)	4 (7.3%)	0	3 (5.3%)	20(5.3%)
Rash	4 (5.2%)	4 (5.2%)	1 (1.8%)	7 (12.7%)	0	3 (5.3%)	19 (5.0%)

Source: GS-US-342-0102, Version 1 TFLs (21 March 2014), Table 27.2

L.5.2 Death

As of 07 April 2014, 1 death was reported in the ongoing Phase 2 studies. The death is described below.

A 36-year-old white male had an extensive psychiatric history that included bipolar disorder, depressive disorder, anxiety, and drug and alcohol abuse. The subject was administered SOF 400 mg + GS-5816 25 mg and committed suicide after completing 12 weeks of treatment. The investigator assessed the event as not related to study drug, but precipitated by acute external events (impending re-incarceration) in the setting of underlying severe psychiatric disease.

L.5.3 Serious Adverse Events

As of 07 April 2014, a total of 19 SAEs were reported in the ongoing Phase 2 studies. Only one was assessed by investigators as being related to study drug. Notable SAEs occurring in one patient each were post-infectious hypersensitivity vasculitis that did

not result in treatment interruption and a seizure in a subject with known seizure disorder felt to be due to non-compliance with anti-seizure medication.

In phase 2 studies including 377 patients, treatment-emergent Grade 3 and 4 laboratory abnormalities were infrequent and are presented in Table 4-27.

There was a higher incidence of Grade 3 hemoglobin decrease and Grade 3 bilirubin increase in ribavirin-treated subjects consistent with ribavirin-induced hemolysis. A total of 4 subjects reported Grade 3 lipase and 1 subject reported Grade 4 lipase; in all cases the lipase elevations were transient and asymptomatic. One subject had a Grade 3 creatinine increase that normalized on treatment and was assessed as being due to dehydration and volume depletion by the investigator. An additional laboratory abnormality that led to treatment interruption was grade 4 elevation in serum ALT.

Table 4-27. GS-US-342-0102: Treatment-Emergent Grade 3 or 4 Laboratory Abnormalities

	Groups 1, 3, and 5 SOF 400 mg + GS-5816 25 mg 12 Weeks (N = 77)	Groups 2, 4, and 6 SOF 400 mg + GS-5816 100 mg 12 Weeks (N = 77)	Groups 7 and 11 SOF 400 mg + GS-5816 25 mg 8 Weeks (N = 56)	Groups 8 and 12 SOF 400 mg + GS-5816 25 mg + RBV 8 Weeks (N = 55)	Groups 9 and 13 SOF 400 mg + GS-5816 100 mg 8 Weeks (N = 55)	Groups 10 and 14 SOF 400 mg + GS-5816 100 mg + RBV 8 Weeks (N = 57)	Total (N = 377)
Subjects with Grade 3 and 4 Laboratory Abnormalities	4 (5.2%)	2 (2.6%)	2 (3.6%)	6 (10.9%)	0	9 (15.8%)	23 (6.1%)
Grade 3							
Total Bilirubin (Hyperbilirubinemia)	0	0	0	1 (1.8%)	0	1 (1.8%)	2 (0.5%)
Creatinine	1 (1.3%)	0	0	0	0	0	1 (0.3%)
Lipase	3 (3.9%)	1 (1.3%)	0	0	0	0	4 (1.1%)
Neutrophils	0	0	0	0	0	1 (1.8%)	1 (0.3%)
Glucose	0	1 (1.3%)	1 (1.8%)	0	0	0	2 (0.5%)
Hemoglobin	0	0	0	6 (10.9%)	0	7 (12.3%)	13 (3.4%)
Grade 4							
Lipase	0	0	1 (1.8%)	0	0	0	1 (0.3%)

Source: GS-US-342-0102, Version 1 TFLs (21 March 2014), Table 33

L.5.4 The risks and hazards of sofosbuvir and GS-5816 combination therapy safety conclusions

As of 07 April 2014, a total of 1167 subjects have been administered GS-5816, of which 872 were subjects with chronic HCV infection.

GS-5816 was well tolerated in Phase 1 studies at doses up to 450 mg for 7 days. There were no deaths or SAEs reported in any Phase 1 studies to date.

Phase 2 safety data from over 800 subjects in Phase 2 studies indicates that SOF 400 mg + GS-5816 25 mg ± RBV or SOF 400 mg + GS-5816 100 mg ± RBV for 8 or 12 weeks was well tolerated. The most frequently reported AEs were fatigue, headache, nausea,

insomnia, and upper respiratory tract infection. One subject death (suicide) was reported in a subject with extensive psychiatric medical history. There was a low rate of treatment discontinuation for AEs. There was a low incidence of SAEs, and severe or life-threatening AEs, and Grade 3 and 4 laboratory abnormalities.

L.6 The risks and hazards of lymphaphoresis

This section applies only for subjects who qualify and agree to undergo a lymphaphoresis procedure. Lymphaphoresis refers to the separation and removal of large numbers of white blood cells (lymphocytes) from the rest of the circulating blood, using an automated cell separator or apheresis device. Your pulse, blood pressure and temperature will be taken, and then you will be asked to lie on a recliner or bed. Your blood will be withdrawn using a needle placed in one arm and directed into a cell separator machine, which separate the blood cells by spinning.

The white blood cells will be retained in a plastic bag inside the machine, while your red cells and plasma are returned to you through the same needle. A short-acting blood thinner named citrate is given to you through one of the needles to prevent your blood from clotting while it is in the machine. Depending on how many white cells are collected, the procedure may last from one to two hours. The number of white cells collected is a tiny fraction of the total number of these cells in your body. They are quickly replaced by your body. The procedure will be performed in the Apheresis Clinic of the NIH Department of Transfusion Medicine (Blood Bank).

The major risks and hazard of undergoing a lymphaphoresis procedure include pain and bruising at the needle placement site. As with other kinds of blood drawing, temporary lowering of the blood pressure may occur, and lightheadedness, dizziness and even fainting may result from this. Use of the anticoagulant may cause tingling sensations around the mouth, fingers, and toes, chills, nausea, heartburn, and mild muscle cramps. These symptoms are easily treated by temporarily interrupting the procedure. Very rarely, there may be loss of as much as one pint of blood due to machine malfunction. Because lymphaphoresis may affect how certain medications work, the procedure will not be performed in subjects receiving interferon.

M. Safety Assessments

Adverse events will be evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events.

N. Toxicity Management

Due to a clinical or laboratory event, administration of all study drugs(s) may be discontinued. There is no option for SOF/GS-5816 FDC dose reduction. If SOF/GS-5816 FDC is stopped due to toxicity, it will not be restarted; if SOF/GS-5816 FDC is discontinued, the subject must complete an ET visit. Post Treatment 4, 8, 12 and 24-Week visits will be scheduled from the last dose of study drug.

O. Adverse Event Reporting

O.1 Reporting to the IRB

Adverse events, protocol deviations, unanticipated problems (UP), Unanticipated Adverse Device Effects (UADEs), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 (“Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations.”). All adverse events at least possibly related to the patient’s participation in the research protocol, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event.

Non-serious protocol deviations will only be reported to the IRB (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues such as those associated with sampling that do not impact the health of the subject or the interpretation of the study data will not be reported.

Adverse events that are expected as a part of treatment or procedures outlined in the protocol will not be reported to the IRB unless they occur at a rate or severity greater than known to occur in patients undergoing the treatment or procedures. If AEs associated with the treatment and procedures in the study occur at a rate or severity greater than known to occur, they will be reclassified as a UP and reported as such. AEs with known relation to the natural history of chronic hepatitis C or to other pre-existing conditions will not be reported unless they occur at a rate or severity greater than known to occur in patients with hepatitis C or the subject’s other pre-existing conditions. AEs that are unrelated to the research will not be reported. The PI is responsible for summarizing all reportable serious adverse events and adverse events at least possibly related at the time of Continuing Review. Deaths will be reported to the Clinical Director and IRB within 7 days after the PI first learns of the event.

O.2. Reporting to the FDA

The sponsor will notify the FDA by telephone and/or facsimile of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information. This should be followed with a written report as specified by the FDA but no later than 15 days after the sponsor's initial receipt of the information using the MedWatch Form 3500a.

Other Serious and Unexpected Suspected Adverse Reactions (SUSARs) will be reported to the FDA in writing using the MedWatch Form 3500a no later than 15 calendar days after the sponsor's initial receipt of the information.

The following safety information will be summarized by the PI in annual reports to the FDA:

- All adverse events thought to be possibly, probably, or definitely related to study drug will be summarized, tracked, and reported to the FDA at the time of the annual report. Any adverse events with known relation to the natural history of the disease or to other pre-existing conditions will not be reported. Any accident, which results in an AE and is unrelated to the protocol, will not be reported.
- All SAEs will be reported in a summary format to the FDA at the time of the annual report. SAEs that arise after the Informed Consent is signed until 30 days after the last dose of study drug will be reported.
- All SUSARs submitted to the FDA during the past year will be summarized.
- A list of subjects who died during study participation with the cause of death for each subject.
- A list of subjects who withdrew or were withdrawn from the study during the past year with the reason for their withdrawal.
- Any new information available to the study investigator/sponsor that pertains to the risk benefit profile of the study drug.

O.3 Reporting to Gilead

Gilead Sciences is required to expedite to worldwide regulatory authorities reports of SAEs, Serious Adverse Drug Reactions (SADRs) or Suspected Unexpected Serious Adverse Reactions (SUSARs) in line with relevant legislation, including the applicable US FDA Code of Federal Regulation, the European Commission Clinical Trials Directive (2001/20/EC); therefore, Gilead Sciences will be notified immediately regarding the occurrence of any SAE or SADR that occurs after the subject consents to participate in the study, including SAEs/SADRs resulting from protocol-associated procedures as defined in relevant legislation including 2001/20/EC. For fatal or life-threatening events, the investigator will also e-mail or fax copies of hospital case reports, autopsy reports, and other documents when requested and applicable. Transmission of such documents should occur with Personal Subject Details de-identified, without losing the traceability of a document to the Subject Identifiers. Gilead Sciences may request additional information from the investigator to ensure the timely completion of accurate safety reports.

All SAEs and deaths will be reported to Gilead within 24 hours (but in no case later than seven (7) calendar days (for a death or life-threatening event) or fifteen (15) days (for all other SAEs) of the investigator's knowledge of the event. SAEs will be reported to:

Gilead Sciences DSPH: fax 1-650-522-5477
 email Safety_FC@gilead.com

P. Protocol Monitoring Plan

Study procedures will be subject to audits and/or monitoring visits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK quality assurance program plan. Audit and/or monitoring visits results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

Q. Viral Resistance

Resistance selection of HCV subgenomic replicon cells with sofosbuvir has identified the S282T mutation within the HCV NS5B to be associated with reduced susceptibility to sofosbuvir in genotypes 1-5 with EC₅₀ values ranging from 117-346 nM (~2.4-16.2 fold higher compared to wild type EC₅₀ values. S282T mutants remained fully susceptible to GS-5816, GS-9669, GS-9451 and tegobuvir.

To define the resistance profile of GS-5816, HCV genotype 1a and 1b replicon cells were treated with various concentrations of GS-5816 for 3 weeks (8, 40, 200, and 1000 × EC₅₀ for genotype 1a, and 2, 4, 6.6, 13, and 33 × EC₅₀ for genotype 1b), which resulted in selection of resistant colonies at each drug concentration (Study PC-281-2013). Individual colonies and pooled colonies were isolated and analyzed for RAVs in the NS5A gene.

In genotype 1a, most resistant clones encoded the single RAVs L31V or Y93H. Other single RAVs included Q30K, L31M and Y93N, although they occurred at much lower frequencies. Double mutations Q30R/Y93H, Q30H/Y93H or L31V/Y93H were observed in a small number of clones resistant to GS-5816.

In genotype 1b, all GS-5816-resistant clones and pools encoded at least 2 amino acid substitutions within the NS5A region. Interestingly, unlike what has been observed with LDV and DCV, Y93H was never present as a single mutation and instead always

emerged in combination with other RAVs in NS5A, including L31F, L31M, and L31V, suggesting that GS-5816 has a high barrier to resistance against genotype 1b HCV when compared with LDV and DCV.

Monitoring for mutations in the non-structural 5b polymerase (NS5b) and non-structural 5a gene regions of HCV will be performed on serum samples obtained at baseline, and on all serum samples that have HCV RNA ≥ 15 IU/ml from patients receiving sofosbuvir and GS-5816 therapy. Monitoring for resistance mutations will be performed by Gilead Sciences. Novel mutations will be correlated with the clinical response.

R. Efficacy Assessments

R.1 Primary Efficacy Assessment

The primary analysis will focus on changes in ISG expression in the liver and its association with decline in circulating and hepatic HCV RNA concentrations by week 4. The degree of hepatic ISG induction after 4 weeks of therapy relative to that present in the baseline liver biopsy will be evaluated.

R.2 Secondary Efficacy Assessment

Secondary analyses will be comparisons of very early viral kinetics, (rates of rapid virological response [RVR], end-of-treatment response [ETR]), rates of SVR12 and SVR24 and virological relapse in comparison with expected rates in treatment naïve and experienced patients to peginterferon and ribavirin therapy, measurement of cytokine levels, serum aminotransferase levels, improvement in transient elastography scores, rates of sofosbuvir and GS-5816 resistance, and safety of sofosbuvir and GS-5816 focusing largely on the first 4 weeks and end of therapy compared to baseline.

R.3 Other Assessments (Endpoint Definitions):

R.3.1 RVR: rapid virological response (HCV RNA <LLOQ Target not Detected) at week 4)

R.3.2 ETR: end of treatment response (HCV RNA <LLOQ Target not Detected at week24)

R.3.3 SVR 12: sustained virological response at follow-up week 12.

R.3.4 SVR 24: sustained virological response at follow-up week 24.

R.3.5 Virological breakthrough: a ≥ 1 log₁₀ IU/L increase in HCV RNA level from nadir in two consecutive samples at least one week apart in a patient with an initial virological response while on study medications (in the absence of non-compliance)

R.3.6 Virological relapse: HCV RNA \geq LLOQ level after therapy is stopped in a patient who previously achieved an end-of-treatment virological response.

R.3.7 Definition of on-treatment hepatitis flares: an on-treatment hepatitis flare will be defined as an increase in ALT to ≥ 400 U/L AND >3 times baseline (week 0) value. A severe on-treatment hepatitis flare will be defined as an ALT ≥ 800 U/L.

R.3.8 Failure of therapy: may be due to inadequate virological response, breakthrough, relapse, or a serious adverse event requiring discontinuation of treatment.

S. Statistical analysis

Variables to be analyzed in association with a virological response will include: baseline ALT and HCV RNA level, liver histology, HCV subtype, IL28B genotype and previous response to therapy, whether a partial or null responder and ISG levels. Patients with missing data or who drop out of the study will be considered as treatment failures on an intention to treat basis. For comparison of means, Student's t-test will be used. All reported p-values will be 2-sided.

S.1 Planned Interim Analysis for Futility:

An analysis of the SVR rate will be performed after the first 70 subjects have been followed for 4 weeks. If the SVR rate is $>90\%$ (<7 failures) then protocol modifications will be considered. This cut-off was chosen based on a 95% CI for 6/70 of 3.2%-17.7%. If the failures occur primarily in a few risk groups then those groups will be continued, otherwise the trial will be modified or stopped early.

S.2 Demographics and Baseline Characteristics

The following will be summarized for treated subjects:

- Demographics: age, race, gender, geographic region, ethnicity;
- Disease characteristics at baseline: HCV RNA level, HCV genotype and subtype, IL28B SNP genotype and cirrhosis status, prior response to pegIFN α /ribavirin;
- Physical measurements at baseline; height, weight, body mass index, hip and waist circumference;
- Laboratory tests at baseline;
- Prior medications.

S.3 Analysis plan

To address the primary hypothesis that ISG levels are related to virological relapse, the ISG analysis of the on treatment liver biopsy at week 4 will be correlated with off-therapy virological outcome. To address the hypothesis that ISG levels are related to viral level, the ISG analysis of the on treatment liver biopsy at week 4 will be compared to that of the pre-treatment biopsy. We expect that the majority of ISGs will be down-regulated due to the dramatic reduction in viral load expected with this potent combination of antiviral agents. The analyses will be stratified by HCV RNA level comparing subjects

with HCV RNA levels <LLOQ Target not Detected) to those with HCV RNA levels <LLOQ but target detected.

We will also explore whether there is a difference in ISG levels amongst the 4 genotypes at baseline and after 4 weeks of therapy.

S.4 Analysis methodology

The analysis will be based on standard approaches to analysis of microarray data. Since the software and approaches change frequently in this field, we will provide a broad outline of the approach rather than detailed, specific steps.

- a) We will perform quality control and background normalization of the gene array data.
- b) Expression value estimates will be obtained and quantile normalization and median scaling of the genes will be performed.
- c) A list of genes up- or down-regulated >2-fold with $p > 0.05$ will be prepared using the following procedure:
 1. For each patient, genes up- or down-regulated >2-fold in the second biopsy compared to the first will be identified.
 2. Lists will be prepared of genes that are up- or down-regulated >2-fold for any of the subjects.
 3. A paired t-test will be used to identify genes from this list that have a p value <0.05 (Difference is not equal to zero).
 4. The subset of the genes in (3) that are up- or down-regulated in >50% of patients in groups defined relapse and/or presence of cirrhosis will be identified.
 5. This procedure will be repeated with $p < 0.01$, but the primary analysis will be based on the genes selected using $p < 0.05$.
- d) The false discovery rate (FDR) of the t test will be estimated.

A more detailed statistical analysis plan will be developed prior to the start of data analysis.

T. Enrollment of Children, Women and Minority Individuals

The current protocol excludes children (age limit > 18 years) as sofosbuvir and GS-5816 have not been used in children for the treatment of chronic hepatitis C. Currently peginterferon and ribavirin are approved for use in children. Moreover, chronic hepatitis C is uncommon in children in the U.S., and the requirement for two liver biopsies may be prohibitive.

Chronic hepatitis C is about twice as common among men than women. We expect to include women in the proportions that occur in this disease. Thus, we expect 30% to 40% of patients to be women. The exclusion of women interested in getting pregnant or

who are not able to practice adequate birth control would be the only possible bias introduced in attaining adequate representation of women in this study.

The distribution of minority individuals in our study, Viral Kinetics and Liver Gene Expression in Response to Ribavirin and Peginterferon Therapy of Chronic Hepatitis C, (08-DK-0182), at the NIH Clinical Center has been previously reported to the NIDDK, IRB. In this study 40% of subjects were considered minorities, 8% of whom were Hispanic or Latino, based on self-reported race and ethnicity and NIH, HHS guidelines for defining minority race. Thus, we believe minority individuals will be adequately represented in this study.

U. Additional Potentially Vulnerable Subject Populations

This study does not plan to accrue adult subjects who are pregnant or who may be unable to provide consent as this is a more than minimal risk study. NIH employees are not categorically recruited, but in the event that an eligible subject presents who happens to be an NIH employee, we will follow the guidelines outlined in the inclusion of employees in NIH intramural research studies.

V. Research Use, Storage and Disposition of Human Samples and Data:

Patients will have serum stored from selected time points during this study. These specimens will be used for repeat virological testing and special tests as needed (such as for viral levels, HCV RNA sequencing, HCV resistance testing or measurement of serum levels of cytokines, IL28b testing or interferon induced genes) and for future research. Samples may be used to assess factors associated with response or non-response to antiviral therapy. Liver biopsy tissue may be stored if a biopsy is done and residual tissue is available after samples are taken for routine histological staining and evaluation and ISG determination. ISG determination will be evaluated in the Liver Diseases Branch and the routine histological evaluation by the surgical pathology services of the Clinical Center. Stored serum will be used to perform in vitro antiviral susceptibility and cross resistance testing in selected patients who develop virological breakthrough and antiviral resistance. Gilead Sciences will conduct these assays and serum samples will not contain any patient identifiers. Samples will be sent as soon as virologic breakthrough is identified and Gilead Sciences will have 6 months after receipt of the sample in which to provide LDB, NIDDK with the results. Research records and data as well as liver biopsy slides, biopsy reports, liver tissue and sera with the patient's name and a unique identifier will be stored indefinitely in our locked offices and freezers, the medical record department and the pathology department. These materials will be protected and tracked by standard operating procedures in the medical record and pathology departments as well as a compulsive filing system in our locked offices and freezers. There will be redundant storage of clinical information in the medical record department and our offices. Likewise, there will be redundant storage of biopsy information and materials in the pathology department and our offices. This should minimize the risk of loss or destruction of information and specimens. If that were to occur we would report it to the

IRB. We do not plan to destroy this personal medical information or the liver biopsy specimens or research subject sera or cryopreserved PBMC after completion of the study because it may be critically important for physicians (here or elsewhere) to have access them when caring for these patients in the future.

W. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be approved by Gilead Sciences before implementation. All protocol modifications must be submitted to the IRB in accordance with local requirements. Approval must be obtained before changes can be implemented.

References:

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005;5:558-67.
2. Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009;29 Suppl 1:74-81.
3. Esteban JI, Sauleda S, Quer J. The changing epidemiology of hepatitis C virus infection in Europe. *J Hepatol* 2008;48:148-62.
4. Wasley A, Alter MJ. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin Liver Dis* 2000;20:1-16.
5. Kamal SM, Nasser IA. Hepatitis C genotype 4: What we know and what we don't yet know. *Hepatology* 2008;47:1371-83.
6. Armstrong GL, Wasley A, Simard EP, et al. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006;144:705-14.
7. Davis GL, Alter MJ, El-Serag H, et al. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology* 2010;138:513-21, 521 e1-6.
8. Ly KN, Xing J, Klevens RM, et al. The increasing burden of mortality from viral hepatitis in the United States between 1999 and 2007. *Ann Intern Med* 2012;156:271-8.
9. Ghany MG, Strader DB, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009;49:1335-74.
10. Ghany MG, Nelson DR, Strader DB, et al. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011;54:1433-44.
11. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;364:2405-16.
12. Poordad F, McCone J, Jr., Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;364:1195-206.
13. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013;368:1878-87.
14. Manns M, Marcellin P, Poordad F, et al. Simeprevir with pegylated interferon alfa 2a or 2b plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-2): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2014;384:414-26.
15. Jacobson IM, Dore GJ, Foster GR, et al. Simeprevir with pegylated interferon alfa 2a plus ribavirin in treatment-naïve patients with chronic hepatitis C virus genotype 1 infection (QUEST-1): a phase 3, randomised, double-blind, placebo-controlled trial. *Lancet* 2014;384:403-13.
16. <http://www.hcvguidelines.org/>. Recommendations for Testing, Managing, and Treating Hepatitis C.
17. Everson GT, TT, Towner, WJ, Davis, WN, Wyles, D, Nahass, R, McNally, J, Brainard, DM et al. . Safety and Efficacy of Treatment with the Interferon-Free, Ribavirin-Free Combination of Sofosbuvir + GS-5816 for 12 Weeks in Treatment-Naïve Patients with Genotype 1–6 HCV Infection. Abstract #O-111.

- Presented at: The International Liver Congress 2014; April 9-13, 2014; London. 2014.
18. Szabo G, Bala S. MicroRNAs in liver disease. *Nat Rev Gastroenterol Hepatol* 2013;10:542-52.
 19. Gilad S, Meiri E, Yogev Y, et al. Serum microRNAs are promising novel biomarkers. *PLoS One* 2008;3:e3148.
 20. Brase JC, Wuttig D, Kuner R, et al. Serum microRNAs as non-invasive biomarkers for cancer. *Mol Cancer* 2010;9:306.
 21. Wang K, Zhang S, Marzolf B, et al. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci U S A* 2009;106:4402-7.
 22. Starkey Lewis PJ, Dear J, Platt V, et al. Circulating microRNAs as potential markers of human drug-induced liver injury. *Hepatology* 2011;54:1767-76.
 23. Bala S, Petrasek J, Mundkur S, et al. Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases. *Hepatology* 2012;56:1946-57.
 24. Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 2008;4:77-85.
 25. Jopling CL, Yi M, Lancaster AM, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005;309:1577-81.
 26. Janssen HL, Reesink HW, Lawitz EJ, et al. Treatment of HCV infection by targeting microRNA. *N Engl J Med* 2013;368:1685-94.
 27. Mancone C, Steindler C, Santangelo L, et al. Hepatitis C virus production requires apolipoprotein A-I and affects its association with nascent low-density lipoproteins. *Gut* 2011;60:378-86.
 28. Sorrentino V, Nelson JK, Maspero E, et al. The LXR-IDOL axis defines a clathrin-, caveolae-, and dynamin-independent endocytic route for LDLR internalization and lysosomal degradation. *J Lipid Res* 2013;54:2174-84.
 29. Syed GH, Tang H, Khan M, et al. Hepatitis C virus stimulates low-density lipoprotein receptor expression to facilitate viral propagation. *J Virol* 2014;88:2519-29.
 30. Butt AA, Yan P, Simon TG, et al. Changes in circulating lipids level over time after acquiring HCV infection: results from ERCHIVES. *BMC Infect Dis* 2015;15:510.
 31. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL Particle Number and Risk of Future Cardiovascular Disease in the Framingham Offspring Study - Implications for LDL Management. *J Clin Lipidol* 2007;1:583-92.
 32. Jung HJ, Kim YS, Kim SG, et al. The impact of pegylated interferon and ribavirin combination treatment on lipid metabolism and insulin resistance in chronic hepatitis C patients. *Clin Mol Hepatol* 2014;20:38-46.
 33. National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-421.

Appendix I

Ancillary Study I

Natural killer (NK) and HCV-specific T cells are an important component of the innate and adaptive immune response and play a role in the outcome of HCV infection. Both NK cells and HCV-specific T cells have been shown to be impaired in their function (in particular in the production of IFN-gamma) in chronic HCV infection.

For T cells this impairment has been attributed to chronic T cell receptor stimulation resulting in upregulation of inhibitory molecules such as PD-1, Tim-3 and CTLA-4 – the molecular signature of a functionally “exhausted” T cell phenotype (reviewed in Rehermann, B, *Nat Med.* 2013 Jul;19(7):859-68). For NK cells this impairment has been attributed to direct exposure to HCV proteins (*J Exp Med.* 2002 Jan 7;195(1):35-41, to HCV-infected hepatocytes (*J Virol.* 2011 Dec;85(23):12557-69) and to chronic exposure to HCV-induced endogenous interferon-alpha which thwarts their function from IFN-gamma production to predominantly cytotoxicity (Rehermann et al., *Gastroenterology.* 2010 Jan;138(1):325-35).

It is also known that a sustained virological response to interferon-based therapy does not restore HCV-specific T cell function and NK cell IFN-gamma production (Rehermann et al., *Hepatology.* 2004 Jul;40(1):87-97; Rehermann et al., *Gastroenterology.* 2011 Oct;141(4):1231-9) and this may impact immunosurveillance of viral relapse and longterm immunity against reinfection. Interestingly, it has recently been shown that HCV-specific T cell responses recover during an interferon-free regimen of direct acting antivirals (Robert Thimme, U of Freiburg, Germany, personal communication, Nov 2013).

This ancillary immunological study will answer the following two questions:

1. AIM 1: What is the mechanism underlying the recovery of HCV-specific T cell responses? We will differentiate between the possibility that HCV-specific T cells with an exhausted functional and molecular phenotype re-gain their function and convert into memory T cells and the alternative possibility that a small subset of not-exhausted and not functionally impaired HCV-specific T cells, that is below the detection limit of immunological assays in chronic infection, has a growth advantage becomes the dominant population when the inhibitory effects of HCV are removed by successful interferon-free antiviral therapy. The distinction between both possibilities is important because to date it is believed that an exhausted T cell phenotype is irreversible. This is relevant not just for HCV, but also for HIV and cancer immunology, where exhausted T cell responses are observed. If reversion of an exhausted phenotype can be observed at the single cell level, we will analyze the molecular mechanisms that govern the change in T cell differentiation and expression of inhibitory molecules.

These studies require prospectively collected lymphocytes to follow changes in T cell markers and function throughout the study using a panel of ex vivo and in vitro immunological assays (see below as described under aim 2). This study will be limited to the genotype 1 infected patients (n=40) because the most comprehensive set of immunological reagents (peptides, T cell epitopes, MHC class I tetramers) is available for the HCV genotype 1 sequence. About 50% of these genotype 1-infected patients (selected based on expression of HLA-A2) will be asked to donate a larger number of lymphocytes (isolated from either lymphopheresis or 100 ml of full blood) from a time point before and a time point after HCV clearance, so that HCV-specific CD4 and CD8 T cells can be sorted by flow cytometry using HLA-A2/HCV peptide tetramers and analyzed on a single cell basis for the underlying molecular mechanisms. The large number of lymphocytes is required because CD8 T cells that are specific for any given HCV epitope constitute about 0.02% to 0.5% of the CD8 T cells in the blood and CD4 T cells that are specific for any given HCV epitope are about 100-fold less frequent.

The following biospecimens are required (genotype 1 patients only):

Blood:

Screening visit: 40ml of heparin-blood from all genotype 1 patients

Prior to treatment: HLA class I and II typing of genotype 1 patients

After HLA classification, prior to treatment: double-pass lymphopheresis (preferred) or 100 ml of blood only from HLA-A2+ genotype 1 patients.

If pre-treatment biopsy is more than one week before start of treatment, at biopsy: 40 ml of heparin-blood from all genotype 1 patients.

Day 0 before initiation of treatment, week 2, week 4, week 12 on treatment: 40 ml of heparin-blood from all genotype 1 patients irrespective of HLA-type

Week 12 (end of therapy): double-pass lymphopheresis (preferred) or 100 ml of blood from HLA-A2+ genotype 1 patients.

Week 24 post end of therapy: 40 ml of heparin-blood from all genotype 1 patients.

Liver biopsy:

A small (5 mm) fragment in RPMI/10% FCS for analysis of intrahepatic NK and T cell responses pre-treatment and at week 4 of therapy.

2. AIM 2: Does restoration of T and NK cell function that follows the DAA-induced decrease in HCV titer improve immune surveillance of small traces of persisting virus thereby decreasing the risk of a virological breakthrough? Can T cell and NK function be used as a predictor of virological relapse at time points at which HCV RNA is undetectable in the blood by standard molecular assays.

This immunological study will be conducted with blood and liver biopsy specimens from genotype 3 infected patients, because a high (33%) chance of virological relapse is expected in this group, and in genotype 2 and 4-infected patients, because the chance of virological relapse is still unknown for these groups. Immunological testing will include cytotoxicity and cytokine production of NK cells and proliferation and cytokine production of CD4 and CD8 T cells in response to HCV proteins and overlapping peptides covering the entire HCV genome. As for aim 1 all tests will be performed in the section of Dr. Barbara Rehermann, Liver Diseases Branch, on the following samples:

The following biospecimens are required (genotype 2, 3 and 4 patients only).

Blood:

Screening visit: 40ml of heparin-blood

If pre-treatment biopsy is more than one week before start of treatment, at biopsy: 40 ml of heparin-blood.

Day 0 before initiation of treatment, week 2, week, 4, week 12 on therapy, and post-therapy visits week 2, 4, 8, 12, 24: 40 ml of heparin-blood .

Liver biopsy:

A small (5 mm) fragment in RPMI/10% FCS for analysis of intrahepatic NK and T cell responses pre-treatment and at week 4 of therapy.

Ancillary Study II

Chronic HCV infection is intimately involved with lipid metabolism of the cell. HCV exploits the host lipoprotein machinery to complete many phases of its life cycle from viral entry to virion production.²⁷⁻²⁹ As a result of this interaction, chronic HCV infection may lead to a relative hypolipidemia with reduced low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), serum triglycerides and total serum cholesterol.³⁰ This observation suggests that sustained eradication of HCV might lead to dyslipidemia (defined as an elevated serum cholesterol with low HDL-C and elevated LDL-C,) and increased risk for cardiovascular disease.

Indeed, follow-up studies after successful clearance of HCV with pegylated interferon-based therapy have reported increases in LDL-C.³² However, these results may have been influenced by changes in weight, thyroid dysfunction and hypertriglyceridemia related to therapy with pegylated interferon. Thus, the true impact of successful eradication of HCV on lipid and lipoprotein levels has not been clearly defined. The availability of very potent and safe direct acting antivirals are not associated with changes in weight or endocrine function and would permit a more unbiased assessment of changes in lipid and lipoprotein levels during and after successful HCV treatment.

Dyslipidemia, characterized by elevated serum cholesterol with low HDL-C and elevated LDL-C, is a major contributor to development of atherosclerotic diseases including stroke, coronary artery disease (CAD) and peripheral vascular disease.³³ More recently it has been shown that higher LDL particle number is strongly associated with cardiovascular risk. Therefore this study will aim to characterize the changes in serum lipids and lipoproteins during and after therapy with direct acting antiviral agents and assess cardiovascular risk following eradication of HCV based on serum/plasma LDL particle number.

Specific hypotheses to be tested:

- 1) Suppression of HCV viremia will lead to rapid changes (with 24-72 hours) in serum/plasma cholesterol, LDL and LDL particle number
- 2) Sustained eradication of HCV will result in increased cardiovascular risk as determined by an increase in LDL particle number

Specific Aims:

Aim 1: To characterize changes in serum/plasma lipids and lipoproteins and its relation to HCV viral kinetics in among subjects with CHC genotype 1-4 during therapy with sofosbuvir and GS5816.

Aim 2: To characterize changes in serum/plasma lipids and lipoproteins after successful eradication of HCV among subjects with genotype 1-4 following therapy with sofosbuvir and GS5816 (post-treatment weeks 12 and 24).

Aim 3: An exploratory aim will be to assess whether subjects with pre-existing dyslipidemia on lipid lowering therapy will require an increase in therapy following eradication of HCV.

The analysis will control for weight and insulin sensitivity. Insulin resistance will be calculated using fasting Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Hemoglobin A1c.

The schedule for additional tests is shown on the table below:

	Pre-Rx	On Treatment							Post-Treatment		
	Pre-Rx liver biopsy	Start of Rx	24 hours	48 hours	72 hours	Week 1	Week 4	Week 12	Week 4	Week 12	Week 24
Serum/Plasma lipid panel	x	x	x	x	x	x	x	x	x	x	x
Serum/plasma lipoprotein	x	x	x	x	x	x	x	x	x	x	x
Serum insulin	x					x	x	x	x	x	x
Hgb A1C	x						x	x		x	x

Additional blood draws will be limited to lipoprotein levels since serum/plasma lipid and insulin levels can be evaluated from existing blood obtained for hepatic and mineral panels and Hgb A1C can be assessed from blood drawn for CBC. The increase in blood volume is estimated to be 33 ml over 9 month. The total amount of blood drawn during an 8-week period will not exceed 10.5 ml/Kg (550 mls).

Appendix III

Package insert for sofosbuvir

Appendix IV

IB for GS-5816

Appendix V

IB for Sofosbuvir and GS-5816