The HepQuant SHUNT Test for Monitoring Liver Disease and Treatment Effects by Measuring Liver Function and Physiology

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Author: Gregory T. Everson, MD

Clinical Investigator and Clinical Testing Site

Study Title: The HepQuant SHUNT Test for Monitoring Liver Disease and Treatment Effects by Measuring Liver Function and Physiology		
Principal Investigator:		
Dr R Bhamidimarri, Dr N Alkhouri, Dr A Sanyal, Dr R Reddy, Dr M Fuchs, Dr K Bhamba, Dr P Mantry, Dr J Trotter		
Dr S Harrison, Dr M Waters		
Sponsor:	HepQuant LLC	
Device:	HepQuant SHUNT Liver Diagnostic Kit	
Funding and		
Investigational device		
Provided by:	HepQuant LLC	
Indication:	Monitoring Liver Disease and Treatment Effects	
Parallel Drug Trials:	GS-US-384-1943 / Gilead Sciences;	IND 129570
	GS-US-384-1944 / Gilead Sciences;	IND 129570
	GS-US-454-4378 / Gilead Sciences;	INDs Pending
Compounds used in HepQuant SHUNT Test Kit:	[24-^{13}C]cholic acid (13C-cholate or 13C-CA)	
	[2,2,4,4-^{2}H(D)]cholic acid (4D-cholate or 4D-CA)	
Drug used in the Clinical Drug Trials:	Selonsertib; GS-0976; GS-9674	
IDEs (HepQuant):	G170034-S001 for GS-US-384-1943 (NASH F3, STELLAR 3)	
	G170034-S002 for GS-US-384-1944 (NASH F4, STELLAR 4)	
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Participating institutions:		
Schiff Center for Liver Diseases at the University of Miami		
Texas Liver Institute		

Virginia Commonwealth University
University of Pennsylvania
Hunter Holmes McGuire VA Medical Center
University of Washington
Methodist Dallas
Baylor University
eStudy Site
Pinnacle Clinical Research

IDE Compliance: This study will be conducted under US Food & Drug Administration IDE regulations (21 CFR Part 812).

GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

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Protocol Synopsis

Study Title:	**The HepQuant SHUNT Test for Monitoring Liver Disease and Treatment Effects by Measuring Liver Function and Physiology**	
Sponsor:	HepQuant LLC	
Device:	HepQuant SHUNT Liver Diagnostic Kit	
Funding BY:	HepQuant LLC	
Device Provided by:	HepQuant LLC	
Protocol:	**ID:**	HQ-US-SHUNT-1701
	Version:	Original Version 5.0
		Amended Version 6.0
	Date:	Original date September 5, 2017
		Amendment date May 15, 2018
Compounds used in HepQuant SHUNT Test Kit:		[24-^{13}C]cholic acid (13C-cholate or 13C-CA)
		[2,2,4,4-^{2}H(d4)]cholic acid (d4-cholate or d4-CA)
Indication for Use:	Monitoring effects of Liver Disease and Treatment on Liver Function	
Gilead Parallel Clinical Trials:	GS-US-384-1943 (STELLAR 3) / Gilead Sciences:	IND 129570
	GS-US-384-1944 (STELLAR 4) / Gilead Sciences;	IND 129570
	GS-US-454-4378 (NASH F3/F4) / Gilead Sciences;	INDs pending
Drug used in the Parallel Drug Trials:	Selonsertib, GS-0976, and GS-9674	
IDE Numbers (for use of the HepQuant SHUNT Test Kit in subjects co-enrolled in the Parallel Drug Trials):		
	G170034-S001 for GS-US-384-1943 (STELLAR 3)	
	G170034-S002 for GS-US-384-1944 (STELLAR 4)	
	IDE pending for GS-US-454-4378 (NASH F3 / F4)	
Study Centers/PIs: This HepQuant SHUNT study is run in parallel to three Gilead clinical trials. Subjects are simultaneously participating in this HepQuant SHUNT study while participating in a Gilead-sponsored clinical trials for treatment of NASH. Because the HepQuant study will focus on the subjects with more advanced liver disease, the clinical sites participating in the HepQuant study must also be participating in one of the NASH F3 (STELLAR 3), NASH F4 (STELLAR 4), or GS-US-454-4378 (NASH F3 and F4) studies. The maximum number of clinical sites is 15 and the maximum number of study subjects is 100.		

Primary Objective: To determine the utility of the Disease Severity Index (DSI) from the HepQuant SHUNT test for monitoring liver disease and treatment effects, DSI and the change in DSI (ΔDSI) will be measured across all treatment arms.		
The clinical significance of ΔDSI will be evaluated by analyzing the relationships of ΔDSI to changes in standard laboratory tests, clinical models, histologic stage of disease, and risk for clinical outcome.		
We will also evaluate whether the HepQuant SHUNT test and DSI identify a treatment effect in the combined NASH F3 + NASH F4 cohorts, after unblinding of treatment assignments and completion of the primary analysis for Gilead's clinical trials. Treatment effect, as measured by DSI and ΔDSI, will be defined from the differences in DSI between treatment and placebo arms; and, compared to the treatment effect defined by changes in histology, standard laboratory tests, clinical models and by clinical outcomes.		
Secondary Objectives: To determine the ability of baseline DSI, performed prior to treatment, to assess the severity of liver disease by: 1. correlation with other baseline tests of liver disease severity; and, 2. prediction of risk for future clinical outcome.		
Study Design:	Open Label HepQuant SHUNT Testing in All Subjects	
	STELLAR 3:	Baseline and Weeks 24, 48, and 240
	STELLAR 4:	Baseline and Weeks 24, 48, and 240
	GS-US-454-4378 (NASH F3 / F4):	Baseline and Weeks 24 and 48
Subjects, N:	Up to a total of 100 for all trials	
Clinical Sites, N:	Up to 15	
Target Populations:	NASH F3:	NASH Clinical Research Network (CRN) Fibrosis Stage 3
	NASH F4:	NASH CRN Fibrosis Stage 4 (compensated cirrhosis)

Compounds used in HepQuant SHUNT Test Kit:	[24-^{13}C]cholic acid, 20 mg, intravenous injection
	[2,2,4,4-^{2}H(D)]cholic acid, 40 mg, oral solution

Drug Treatment:	STELLAR 3:	Selonsertib vs Placebo
	STELLAR 4:	Selonsertib vs Placebo
	GS-US-454-4378 (NASH F3/F4): Selonsertib, GS-0976, GS-9674 alone or in combination versus placebo.	
Duration of Drug Treatment:	STELLAR 3:	240 weeks
	STELLAR 4:	240 weeks
	GS-US-454-4378 (NASH F3/F4):	48 weeks
Duration of Study and Drug Trial:	STELLAR 3:	252 weeks
	STELLAR 4:	252 weeks
	GS-US-454-4378 (NASH F3/F4):	52 weeks

Diagnostic and Eligibility Criteria:	Enrollment in the corresponding Gilead Trial, listed above	
	Intravenous access for catheter placement	
	Able to take orally administered solution	
	No known allergy to any component of the HepQuant SHUNT test kit	
Procedures and Frequency of the HepQuant SHUNT Testing:		
	STELLAR 3:	Baseline and Weeks 24, 48, and 240
	STELLAR 4:	Baseline and Weeks 24, 48, and 240
	GS-US-454-4378 (NASH F3/F4):	Baseline and Weeks 24 and 48
Dose and Mode of Administration of Drug used in Drug Trial:		
	STELLAR 3 and 4:	Selonsertib 6 or 18 mg orally daily
	GS-US-454-4378:	Selonsertib 18 mg, GS-0976 20 mg, or GS-9674 30 mg for both monotherapy and combinations; Orally; Daily dosing
Reference Therapy in Drug Trial, Dose, Mode of Administration:	Placebo tablets; Orally; Daily	
Criteria for Evaluation (HepQuant test parameters are the same for all studies):		
	Baseline DSI:	DSI measured prior to randomization
	ΔDSI_i:	Change in DSI from baseline to time point, i
		Treatment Effect: ΔDSI_i Treatment versus ΔDSI_i Placebo
Primary Clinical Outcomes to link to baseline DSI and ΔDSI_i:		
STELLAR 3:	Wk48:	1 Stage improvement in fibrosis without worsening NASH
	Wk48:	Freedom from progression to cirrhosis
	Wk48:	Freedom from decompensation, transplant, or death
	Wk240:	1 Stage improvement in fibrosis without worsening NASH
	Wk240:	Freedom from progression to cirrhosis
	Wk240:	Freedom from decompensation, transplant, or death
STELLAR 4:	Wk48:	1 Stage improvement in fibrosis without worsening NASH
	Wk48:	Freedom from decompensation, transplant, or death
	Wk240:	1 Stage improvement in fibrosis without worsening of NASH
	Wk240:	Freedom from decompensation, transplant, or death
GS-US-454-4378:	Endpoints as above for F3 and G] F4 cases but only for 48 Weeks	

Statistical Methods:

We will determine the utility of the Disease Severity Index (DSI) from the HepQuant SHUNT test to monitor liver disease and treatment effects by measuring liver function and physiology. We propose a three-step analytical process to achieve this goal. First, without knowledge of treatment arm, we will track DSI and the change in DSI, ΔDSI, from baseline to on-treatment and follow-up timepoints. Second, we will apply separate survival models to evaluate ΔDSI as a continuous variable and a 2 point or more change in DSI as dichotomous variable in the prediction of changes in other tests or models of liver disease severity and clinical outcomes. In HCV, an increase in DSI of 2 or more has been associated with disease progression; and, a decrease in DSI of 2 or more has been associated with improvement after viral clearance. Third, once the treatment arms have been unblinded we will evaluate if the use of the HepQuant SHUNT test and DSI identify a treatment effect in the combined cohort of NASH F3 plus NASH F4; and we will compare DSI to the primary measures of efficacy in each of the clinical trials. In terms of DSI, a treatment effect will be defined from the difference between the ΔDSI of the treatment arm versus the ΔDSI of the placebo arm. The significance of this difference will be evaluated using a two-sided t-test. Furthermore, we will link treatment effect defined by DSI to effects of treatment on other tests and clinical outcomes (bleeding from varices or portal hypertensive gastropathy, ascites, encephalopathy, SBP, progression to cirrhosis, and patient mortality or liver transplantation). We will use both generalized estimating equations (GEE) and profile analysis – profile of DSI between treatment and placebo groups at each time point – for additional primary endpoint analyses.

We will also determine the ability of baseline DSI, performed prior to treatment, to assess the severity of liver disease by: 1. correlation with other baseline tests of liver disease severity; and, 2. prediction of risk for future clinical outcome.

Correlation of Baseline DSI with other baseline tests:

STELLAR 3:	Liver histology; standard labs; MELD & CP scores
STELLAR 4:	Liver histology, standard labs, MELD & CP scores
GS-US-454-4378:	As above for F3 and F4 disease

In an analysis of the combined NASH F3 and F4 cohorts, we will correlate the baseline DSI with histology, lab tests, and clinical models by linear regression and Spearman's correlation coefficients. Regression analysis will be used to obtain prediction and 95% CI of baseline B-K fibrosis score, standard lab tests, and clinical models based on DSI. In a secondary analysis of the combined NASH trials, we will use a discriminant analysis to compare the ability of baseline DSI to distinguish NASH F3 from NASH F4 to the ability of other tests and models to discriminate F3 from F4.

Baseline DSI as Predictor of Risk for future clinical outcome:

We will perform survival analysis for freedom from clinical outcome using Kaplan-Meier plots. Cox models will be used to estimate hazard ratios corresponding to 1 unit of DSI, controlling for other known risk factors of mortality (age, gender, smoking, etc). We will also apply separate survival models to evaluate DSI as a continuous variable and as above or below mean DSI as dichotomous variable in the prediction of clinical outcomes. In a secondary exploratory analysis of the combined NASH trials, we will use a discriminant analysis to compare the ability of baseline DSI to the ability of other tests and models to predict clinical outcomes.

HepQuant Contact Information

Glossary

β-hCG β-human chorionic gonadotropin
AE adverse event
AH alcoholic hepatitis
ALT alanine aminotransferase (also SGPT)
ANC absolute neutrophil count
APTT activated partial thromboplastin time
AST aspartate aminotransferase (also SGOT)
BID twice a day
BLQ below the limit of quantitation
BMI body mass index
BW body weight
CI Confidence Interval
CLD chronic liver disease
CP Child-Pugh (for clinical/laboratory classification of cirrhosis)
CrCL creatinine clearance
CT Computed Tomography
CPK Creatine phosphokinase
Cr serum creatinine
CRF case report form(s)
CYP Cytochrome P450
dL Deciliter
DNA deoxyribonucleic acid
DSI Disease Severity Index (from HepQuant SHUNT test)
DSPH Drug Safety and Public Health
ECG Electrocardiogram
eCRF Electronic case report form(s)
EOT End of Treatment
EU European Union
FDA (United States) Food and Drug Administration
GCP Good Clinical Practice (Guidelines)
GGT gamma glutamyl transferase
GI Gastrointestinal
GT Genotype (viral)
Hgb Hemoglobin
HgbA1c Hemoglobin A1c
HBsAg hemoglobin surface antigen
HBV Hepatitis B virus
HCC Hepatocellular carcinoma
HCV Hepatitis C virus
HFR Hepatic Filtration Rate
IB Investigator Brochure
ICH International Conference on Harmonisation
IDE Investigational device exemption
IEC independent ethics committee
IMP Investigational Medicinal Product
IND Investigational New Drug (Application)
INR International Normalized Ratio of prothrombin time
IRB institutional review board

IUD intrauterine device
IU international units
IV Intravenous
IWRS interactive web response system
Kg Kilogram
L Liter
LDL low-density lipoprotein
LLN lower limit of the normal range
LLOQ Lower limit of quantification
LLT Lower-Level Term
MCV mean corpuscular volume or mean cell volume
MedDRA Medical Dictionary for Regulatory Activities
mg Milligram
mM Millimeter
MH Mantel-Haenszel
mL Milliliter
min Minute
mmHg millimeters mercury
NAFLD Non-Alcoholic Fatty Liver Disease
NASH Non-Alcoholic Steato-Hepatitis
OTC Over the counter
P-gp P-glycoprotein
PG Pharmacogenomic
PH portal hypertension
PI Protease inhibitor
PO by mouth
QD once daily (use only in tablets)
PK Pharmacokinetic
PSC Primary sclerosing cholangitis
PT prothrombin time
RBC red blood cell count
RBV Ribavirin
RNA ribonucleic acid
RVR rapid virologic response
SAE serious adverse event
SD Standard deviation
SEL selonsertib
SMV Olysio/Simeprevir
SOC Standard of Care
SOF Sovaldi/Sofosbuvir
SOP Standard operating procedure
SUSAR Suspected Unexpected Serious Adverse Reaction
SVR Sustained Virologic Response
SVR 4 Sustained viral response 4 weeks after discontinuation of study treatment
SVR 12 Sustained viral response 12 weeks after discontinuation of study treatment
SVR 24 Sustained viral response 24 weeks after discontinuation of study treatment
TVR Telaprevir
US United States
WBC white blood cell count

1.0 Introduction

1.1 Parallel Study Design

1.1.1 Use of the HepQuant SHUNT Test in Clinical Trials

The HepQuant SHUNT test, which is provided as a HepQuant SHUNT Liver Diagnostic Kit, is a minimally-invasive test of liver function and physiology which has been designated by the FDA as an investigational drug/device combination product. Because the HepQuant SHUNT test is an investigational product, its use in clinical trials requires an investigational device exemption (IDE) issued from FDA. HepQuant has already secured IDEs for performing the HepQuant SHUNT test in subjects enrolled in Gilead's clinical trials. HepQuant's IDE numbers for the Gilead trials are:

- G170034-S001 for the GS-US-384-1943 trial of non-alcoholic steatohepatitis (NASH) fibrosis stage 3 (STELLAR-3),
- G170034-S002 for the GS-US-384-1944 trial of NASH and compensated cirrhosis (STELLAR-4), and
- IDE pending for the GS-US-454-4378 trial of NASH fibrosis stage 3 and NASH fibrosis stage 4.

1.1.2 The HepQuant study as a Parallel Study

In this HepQuant study the HepQuant SHUNT test will be performed in subjects enrolled in the three Gilead trials noted above. The HepQuant study protocol, informed consent, and documents govern the administration, monitoring, and analyses of the HepQuant SHUNT test.

The HepQuant study is to run parallel to the Gilead clinical trials. The time points for the HepQuant SHUNT tests coincide with pre-specified time points within the Gilead clinical trials. Subjects enrolled in GILEAD's STELLAR and GS-US-454-4378 (NASH F3/F4) trials may participate concurrently in this HepQuant sponsored investigational device study at participating US sites only once approved by the applicable IRB/IEC.

1.1.3 Coordination and Sponsorship

The main eligibility criteria for enrollment into the HepQuant study is enrollment into one of the three Gilead clinical trials listed above in Section 1.1.1 and criteria noted in Section 5.2. A participant in the HepQuant study will consent to one of the Gilead clinical trials and the HepQuant study at the same visit or when the subject qualifies for one of the above listed Gilead studies. Enrollment into one of the Gilead clinical trials is required for enrollment into the HepQuant study.

The HepQuant study covers all procedures, oversight, and monitoring related to the HepQuant SHUNT test. HepQuant LLC is the sole Sponsor of the HepQuant study. Gilead has contracted with HepQuant for the results of all the HepQuant tests performed in the subjects enrolled in the Gilead trials.

All other aspects of Gilead's clinical trials are covered under Gilead's main protocols and contracts with clinical sites. Gilead Sciences is the sponsor of the three clinical trials noted above. HepQuant test results will be sent to Gilead and Gilead will conduct all correlation analyses with other parameters being collected in the Gilead protocols. HepQuant will request the results of these analyses from Gilead.

1.1.4 Co-enrollment

Subjects enrolled in this HepQuant study of the diagnostic utility of the HepQuant SHUNT test are co-enrolled in a Gilead clinical trial of treatment with selonsertib. Justification for co-enrollment includes:

- Complementary, not competing, interests and endpoints for the two studies
- Collaboration agreement between HepQuant and Gilead
- Independent informed consents
- Gilead will conduct correlation analysis of study results

Co-enrollment into the two studies does not violate any of the ethical principles of respect for persons, beneficence, and justice that guide research involving human subjects.

1.2 Rationale for Conducting this Study

Why conduct this research study? There is an unmet medical need for a non-invasive or minimally-invasive test that accurately measures liver function and physiology. The HepQuant SHUNT Test is minimally-invasive and measures hepatocyte function and inflow to the liver from the simultaneous clearances (hepatic filtration rates, HFRs) of cholate from systemic and portal circulations. The Test quantifies portal-systemic shunting (SHUNT) and generates a liver disease severity index (DSI) [1]. DSI is a score from 0 (no disease) to 50 (terminal illness) that is a composite of both HFRs and correlates with stage of fibrosis, presence of varices, especially large varices, and risk for future clinical outcomes [2-8]. DSI is the primary output variable from the HepQuant SHUNT test. The HepQuant SHUNT test potentially satisfies the unmet medical need for a minimally-invasive test of global liver function and physiology.

HepQuant believes that DSI from baseline and serial HepQuant SHUNT tests will be useful in defining baseline disease severity and that the change in DSI, ΔDSI, will detect clinically meaningful changes in liver disease and responses to treatment.

1.3 The Gilead Clinical Trials

As stated above, HepQuant believes that baseline DSI and change in DSI, ΔDSI, are useful for monitoring liver disease and treatment effects. HepQuant proposes collaborative parallel studies with pharmaceutical drug trials to validate DSI and ΔDSI for monitoring.

The Gilead trials are double-blind, randomized, placebo-controlled clinical trials – the HepQuant study will be run in parallel and coordinated with three Gilead trials:

1. GS-US-384-1943 (STELLAR-3); subjects with non-alcoholic steatohepatitis (NASH) and bridging (F3) fibrosis
2. GS-US-384-1944 (STELLAR-4); subjects with NASH and compensated cirrhosis (F4)
3. GS-US-454-4378; subjects with NASH and bridging (F3) fibrosis or compensated cirrhosis (F4)

The Gilead IND for selonsertib is 129570. The INDs for GS-0976 and GS-9674 are pending. This is provided to HepQuant for cross-referencing. HepQuant's letters of FDA's issuance of the IDEs for each of the trials are provided in Appendix A.

2.0 Primary and Secondary Objectives

As described in Section 1.2, the Disease Severity Index (DSI) from the HepQuant SHUNT test is a score from 0 to 50 based upon the dual simultaneous clearance of cholates (CAs) from systemic (13C-CA) and portal circulations (4D-CA). DSI is the primary output from the HepQuant SHUNT test; and, both DSI and change in DSI (∆DSI) may be useful in monitoring liver disease and treatment effects.

2.1 Primary Objective

The primary objective is to determine the utility of the Disease Severity Index (DSI) derived from the HepQuant SHUNT test, which measures liver function and physiology, for monitoring liver disease and treatment effects.

- Utility will be defined by measuring DSI and the change in DSI (∆DSI) across all treatment arms. DSI and ∆DSI will be evaluated in real time.

- The clinical significance of changes in DSI will be evaluated by analyzing the relationship of ∆DSI to changes in histologic stage of disease, standard laboratory tests, clinical models, or risk for clinical outcome. Some of the proposed comparative analyses will only be performed after unblinding of treatment arms and completion of analyses for the main Gilead trials and may be conducted by Gilead.

- Treatment effects will only be determined after unblinding the treatment arms and completion of the analyses for the main Gilead trials. In terms of DSI, treatment effects will be determined from the differences in DSI and ∆DSI between treatment and placebo arms. The analyses will be conducted by Gilead.

Clinically-meaningful outcomes. As noted above, the subjects will be followed for clinical outcomes in the Gilead trials. In the case of STELLAR 3 and 4, the follow-up for clinical outcomes (bleeding from varices or portal hypertensive gastropathy, ascites, encephalopathy, liver-related death) will be up to 240 Wks – the ∆DSI associated with development of clinical outcome at both Wk48 and Wk240 will be analyzed. In the case of GS-US-454-4378, the follow-up for clinical outcomes will be for 48 Weeks.

Unblinding Treatment Arms. Each of the Gilead NASH trials is placebo-controlled. Once the allocation code for the Gilead clinical trial is unblinded, HepQuant will request the treatment allocation from Gilead in order to analyze the HepQuant SHUNT results in the context of treatment versus placebo. The ΔDSI of the treatment arm will be compared to the ΔDSI of the placebo arm. We anticipate that the ΔDSI in the placebo arm will either not change reflecting stability, or be positive, reflecting the natural progression of liver disease. If treatment is effective the ΔDSI of the treatment arm should be negative – implying a lowering of disease severity and improvement in liver function and physiology. A treatment effect based on DSI will be determined from comparison of ΔDSI of the treatment and control arms.

2.2 Secondary Objective

The secondary objective is to determine the ability of baseline DSI, performed prior to treatment, to assess the severity of liver disease by:

- correlation with other baseline tests of liver disease severity; and,
- prediction of risk for future clinical outcome.

Tests of liver disease severity to compare to baseline DSI. Standard tests for assessing the severity of liver disease include liver histology (stage of fibrosis), standard blood tests (elevated bilirubin and INR, and reduced albumin and platelet count), and clinical models (MELD and CTP scores). Subjects enrolled in the Gilead trials will undergo a battery of these tests, at baseline. Through requests to Gilead for specific data analyses, HepQuant's baseline DSI will be compared to the baseline results of these other measurements, such as fibrosis score on liver biopsy, clinical laboratory tests and models, and other exploratory tests of stage of fibrosis (elastography [FibroScan, MRE] and biomarkers).

Clinically-meaningful outcomes. The subjects will be followed for clinical outcomes. In the case of F3/F4 NASH, the rates of development of clinical outcomes (progression to cirrhosis, bleeding from varices or portal hypertensive gastropathy, ascites, encephalopathy, liver-related death) will be relatively slow so the follow-up is up to 240 weeks – the baseline DSI associated with development of clinical outcome at both Wk48 and Wk240 will be analyzed.

3.0 Background and Significance

3.1 The HepQuant SHUNT Test

3.1.1 Overall Experience with the HepQuant SHUNT Test

The HepQuant SHUNT test is blood-based, minimally-invasive, well tolerated by subjects, simple to administer, and test administration does not require expensive equipment or technology. Over 1400 tests have been performed in over 650 persons without adverse event related to the HepQuant SHUNT test.

This experience encompasses healthy controls, and subjects with chronic hepatitis C, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), primary sclerosing cholangitis (PSC), hepatocellular carcinoma (HCC), polycystic liver disease, and spans the spectrum from minimal to advanced fibrosis and compensated to decompensated disease [1-21].

3.1.2 Test Administration

The HepQuant SHUNT test is performed after an overnight fast or after at least 5 hours of fasting during the daytime. The HepQuant SHUNT test requires venous access via a standard indwelling intravenous catheter, preferably placed in an antecubital vein. An oral solution of 4D-CA and an injectable solution of 13C-CA mixed with 25% human serum albumin are administered simultaneously. Blood samples are obtained at baseline and at 5±1, 20±2, 45±5, 60±5, and 90±5 minutes after dosing. The serum is separated and sent to the HepQuant laboratory for quantification of concentrations of endogenous cholate, 4D-CA and 13C-CA. Clearances are calculated from AUCs and dose and DSI and SHUNT are derived from the clearances.

3.1.3 Test Analysis and Outputs

The HepQuant SHUNT test outputs are:

- **Systemic HFR.** The intravenous clearance (Cl_{iv}, mL min^{-1}) is defined as the dose/AUC for 13C-CA. The Systemic Hepatic Filtration Rate (Systemic

HFR) is defined as the Cl_{iv} per kg of body weight and is expressed as mL min^{-1} kg^{-1}.

- **Portal HFR.** The apparent oral clearance (Cl_{oral}, mL min^{-1}) is defined as the dose/AUC for 4D-cholate. The Portal Hepatic Filtration Rate (Portal HFR) is defined as the Cl_{oral} per kg of body weight and is also expressed as mL min^{-1} kg^{-1}.

- **SHUNT.** SHUNT, the portal-systemic shunt fraction, is calculated as the ratio Systemic HFR/Portal HFR x 100%.

- **DSI.** The calculation for Disease Severity Index is a proprietary formula derived from Systemic HFR and Portal HFR. DSI 0 implies no hepatic impairment and is based on the means (+3 SDs) for Systemic and Portal HFRs of normal weight healthy volunteers. DSI 50 represents severe hepatic impairment as measured in terminally ill CP (Child-Pugh) C cases with clinically advanced liver disease. Subjects with intermediate severity of liver disease have intermediate DSI scores.

- **STAT.** STAT is defined as the 60 minute d4-CA concentration from the SHUNT test, normalized to an ideal body weight of 75 kg. STAT was found in previous studies to correlate closely with DSI (r2 = 0.88). Utilizing this single blood sample will also be evaluated as a simplified testing approach.

For the purpose of the proposed analyses, DSI is considered the primary output variable from the HepQuant SHUNT test. However, in exploratory analyses we will determine the performance of the other test parameters – Systemic HFR, Portal HFR, SHUNT, and STAT.

3.1.4 The HepQuant SHUNT Liver Diagnostic Kit

The test compounds, 13C-CA and d4-CA, and 25% human serum albumin are included in a HepQuant SHUNT Liver Diagnostic test kit with appropriate Instructions for Use. In brief, the contents of the kit include:

- Sealed vial of sterile solution of d4-CA (40 mg in 10 mL sodium bicarbonate) for oral use

- Sealed vial of sterile solution of 13C-CA (22 mg in 5.5 mL sodium bicarbonate) for intravenous use
- Sealed vial of 20 mL of 25% human serum albumin for intravenous use – 5 mL is added to 5 mL of the 13C-CA solution prior to intravenous injection
- 6 blood collection tubes (blood is allowed to clot and serum separated by centrifugation)
- 6 transfer tubes (for transport of serum to HepQuant designated lab)
- Labels
- Instructions for Use
- Mailer

3.1.5 Unmet Need

The HepQuant SHUNT test fulfills an unmet medical need by providing a minimally-invasive test of global liver function and physiology. With progression, chronic liver disease (CLD) impairs hepatocyte function and the portal circulation - abnormalities which manifest as portal hypertension and portal-systemic shunting. HepQuant SHUNT measures the changes occurring in both the liver's function and the portal circulation.

In recognition of the limitations of standard lab tests, liver biopsy, and HVPG, the trans-NIH Action Plan for Liver Disease Research and the RFA for New Technologies for Liver Disease have clearly stated the need for "non-invasive means of assessing the liver" that would "obviate the need for liver biopsy in diagnosis, staging and grading of liver diseases" and that would "accurately reflect the stage of liver disease and can detect mild to moderate degrees of fibrosis before the onset of cirrhosis". The minimally-invasive HepQuant SHUNT test fulfills most, if not all, of these criteria.

3.1.6 Measuring Hepatocyte Function

For decades, investigators have used tests of hepatic metabolism to quantify hepatic function – most are insensitive, lack sufficient reproducibility, and require complex detection systems or analytical methods. However, none can measure the portal circulation or portal-systemic shunting – the latter being a key determinant of clinical complications of liver disease (varices, ascites and encephalopathy). The 13C-methacetin breath test is currently undergoing evaluation in trials; but it suffers from the same issues as other metabolic tests because it requires purchase of expensive bedside

equipment, and is influenced by gender, age, and use of many prescribed and OTC drugs or medications [22-25].

In head-to-head comparisons, the HepQuant SHUNT test performed favorably in comparison to a battery of metabolic tests in prediction of clinical outcomes. The metabolic substrates used in the metabolic tests as comparators were caffeine, antipyrine, lidocaine-MEGX, galactose, 13C-methionine, and 14C-erythromycin [7,8].

Key differences in the HepQuant SHUNT test, compared to metabolic tests, may explain its superior performance:

- HepQuant SHUNT is a dual clearance method tagging both systemic and portal inflow to the liver, i.e., it is a global clearance test.
- HepQuant SHUNT uses cholate, a compound with multiple hepatic transporters and relatively high first-pass hepatic extraction.
- Because multiple transporters are involved, a single nucleotide polymorphism in one transporter is not likely to influence overall cholate uptake.
- Cholate uptake is not dependent upon hepatocyte metabolism - drugs, medications, or other factors affecting cytochrome P450 metabolism.
- By comparing portal to systemic clearance of cholate, HepQuant SHUNT quantifies portal-systemic shunting, a key variable associated with and reflecting the meaningful clinical outcomes of varices, ascites, and encephalopathy.

3.1.7 Measuring the Portal Circulation

Hepatic inflammation and fibrosis within the sinusoid and space of Disse increase the resistance to sinusoidal perfusion. The increase in hepatic resistance to portal inflow raises portal pressure - as CLD progresses, fibrosis accumulates, further raising hepatic resistance and leading to portal hypertension. The splanchnic bed adapts to the rise in portal pressure by vasodilatation and formation of portal-systemic collaterals leading to portal-systemic shunting [26].

Underlying portal hypertension (PH) is a risk factor for poor outcome in chronic liver disease (CLD). PH can be measured directly by percutaneous puncture of the liver to access the portal vein or by threading a catheter from the jugular vein into the portal vein to measure the hepatic venous pressure gradient (HVPG). Increased portal pressure or

HVPG correlates with risk of cirrhosis, varices, ascites, and decompensation. However, these tests are invasive, time consuming, risky, cumbersome, expensive, require specialized equipment and expertise, and are not embraced by subjects [27-29].

HepQuant SHUNT is a global function test capable of monitoring hepatocellular function, total hepatic perfusion, portal inflow to the liver, and portal-systemic shunting. Similar to HVPG, HepQuant SHUNT assesses the portal circulation. In contrast to HVPG, HepQuant SHUNT accomplishes this minimally-invasively with high patient tolerability and lower cost. These characteristics suggest that HepQuant SHUNT could be a minimally-invasive alternative to HVPG in monitoring the effects of liver disease on the portal circulation. In addition, these characteristics suggest that the HepQuant SHUNT test could be useful for detecting changes in response to treatments or interventions.

3.1.8 Measuring Disease Severity by the HepQuant SHUNT Test

In our prior studies of chronic hepatitis C, DSI correlated with ISHAK and METAVIR stages of fibrosis and predicted likelihood of cirrhosis, varices, and risk for clinical outcome. DSI has performed similarly in subjects with chronic hepatitis C, non-alcoholic fatty liver disease (NAFLD), and primary sclerosing cholangitis [2-21].

3.1.9 Measuring Treatment Effect by the HepQuant SHUNT Test

Serial changes in DSI have been assessed in subjects with chronic hepatitis C, during and after antiviral treatment, and in subjects with PSC. Two studies of HepQuant SHUNT for measuring impact of sustained viral response (SVR) are highlighted, GILEAD-sponsored SOLAR-1 and NIH-sponsored HALT-C. In SOLAR-1, subjects were treated with ledipasvir/sofosbuvir/ribavirin, and in HALT-C, subjects were treated with peginterferon/ribavirin. HepQuant SHUNT was performed at baseline and at weeks 4, 24, 36, and 48 in SOLAR-1; and, at baseline and after approximately 2 years in HALT-C. In the PSC study, subjects underwent HepQuant SHUNT tests at baseline and after 1 year.

In SOLAR-1, DSI improved significantly by week 4. The improvement was greatest in transplant recipients with necro-inflammation and non-cirrhotic stages of fibrosis (ΔDSI -4.4±3.7, $p<0.0001$) and in transplant recipients with cirrhosis (ΔDSI -1.7±2.5, $p<0.03$). DSI did not change in non-transplant subjects with end-stage decompensated disease

(ΔDSI 0.2±2.8, p=NS). Standard laboratory tests, MELD score, and CTP score failed to detect the improvement in liver function at week 4 [20].

The results of SOLAR-1 suggest that the decrease in DSI by week 4 was likely due to improvement in the hepatic microcirculation. First, ALT declined in parallel with the clearance of HCV RNA indicating reduction in necro-inflammation. Second, platelet count increased in LT Fibrosis and LT Cirrhosis, most probably from a reduction in splenic sequestration as hepatic resistance declined and portal hypertension improved. Third, the increase in platelet count correlated with the decrease in DSI. Fourth, although improvement in hepatocyte uptake of cholate might also account for the increases in HFRs and decline in DSI, there was no improvement in blood tests of hepatocyte function, such as bilirubin, albumin and INR. These findings are consistent with the interpretation that early hepatic improvement measured by drop in DSI reflects improvement in the hepatic microcirculation.

Because in CLD, necro-inflammation is a hallmark of the injury, baseline DSI will define the severity of the hepatic impairment at start of treatment. With successful treatment by selonsertib, necro-inflammation should be reversed and be manifested as improved hepatic perfusion and hepatocyte uptake – events that should be detected by drop in DSI from the baseline test. If selonsertib has a very rapid onset of action, we could possibly detect improvement at the earliest time point after initiating treatment.

The serial studies in HALT-C and PSC may not be as relevant to detection of early treatment effect since the follow-up DSI in these studies was either 1 year (PSC) or 2 years (HALT-C) after baseline testing. Nonetheless, the results in HALT-C demonstrated significant improvement in DSI after SVR and worsening of DSI in both untreated subjects and treated subjects who remained infected [4]. The study of DSI in PSC subjects identified 3 progressor groups of PSC subjects, slow, moderate, or rapid, based on DSI relative to age and the change in DSI over one year. The serial changes in DSI over one year confirmed slower progression in the slow progressor group compared to a more rapid rate of progression in the moderate to rapid progressor groups [18].

3.2 Investigational Drugs

3.2.1 Selonsertib (ASK1 Inhibitor)

Selonsertib (GS-4997) is an orally bioavailable inhibitor of the apoptosis signal-regulating kinase 1 (ASK1). Upon oral administration, the ASK1 inhibitor selonsertib targets and binds to the catalytic kinase domain of ASK1 in an ATP-competitive manner, thereby preventing its phosphorylation and activation. This prevents the phosphorylation of downstream kinases, such as c-Jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinase (p38 MAPK). By preventing the activation of ASK1-dependent signal transduction pathways, selonsertib prevents the production of inflammatory cytokines, down-regulates the expression of genes involved in fibrosis, suppresses excessive apoptosis and inhibits cellular proliferation.

Selonsertib undergoes hepatic metabolism and biliary excretion. In a mass balance study of radiolabeled selonsertib, the mean overall recovery of radioactivity was 95%, with recovery primarily in feces (~58%) versus urine (~37%). Radioactivity was eliminated as a combination of metabolites and unchanged parent drug. Selonsertib metabolism involved oxidation, hydrolysis, N-dealkylation, methylation, and glucuronidation with N-dealkylation as a major pathway.

Selonsertib is a very weak P-gp inhibitor and may increase exposures of sensitive P-gp substrates such as digoxin. Selonsertib is not a sensitive substrate of hepatic uptake transporters OATP1B1/1B3 and may be co-administered with OATP1B1/1B3 inhibitors. Because rifampin significantly decreased selonsertib plasma exposure, strong CYP3A4 inducers in combination with selonsertib in long-term clinical studies are excluded. There is no anticipated interaction of selonsertib with the cholates used in the HepQuant SHUNT test.

Selonsertib was evaluated in an open-label Phase 2 study, alone or in combination with the monoclonal antibody simtuzumab (SIM) for 24 weeks, in patients with nonalcoholic steatohepatitis (NASH) and moderate to severe liver fibrosis (fibrosis stages F2 or F3). The data demonstrated regression in fibrosis that was, in parallel, associated with reductions in other measures of liver injury in patients treated with selonsertib. Patients receiving selonsertib demonstrated improvements in several measures of liver disease severity, including fibrosis stage, progression to cirrhosis, liver stiffness (measured by magnetic resonance elastography, MRE) and liver fat content (measured by magnetic resonance imaging (MRI)-proton density fat fraction, PDFF). Data for these efficacy endpoints are summarized in the table below. As no differences were observed between combination and monotherapy, results are presented for selonsertib (18 mg and 6 mg) with/without SIM and for SIM alone. Additionally, patients with fibrosis improvement

demonstrated reductions in hepatic collagen content, liver biochemistry (e.g., serum ALT) and the apoptosis marker, cytokeratin-18, supporting the biological activity of selonsertib.

Endpoint (Week 24)	Selonsertib 18 mg ± SIM	Selonsertib 6 mg ± SIM	SIM
Fibrosis Improvement ≥1 Stage from Baseline*	43% (n=13/30)	30% (n=8/27)	20% (n=2/10)
Progression to Cirrhosis	3% (n=1/30)	7% (n=2/27)	20% (n=2/10)
≥15% Reduction in Liver Stiffness by MRE	20% (n=5/25)	32% (n=7/22)	0% (n=0/7)
≥30% Reduction in Liver Fat by MRI-PDFF	26% (n=8/31)	13% (n=3/24)	10% (n=1/10)

*Fibrosis staged according to the NASH Clinical Research Network (CRN) classification.

There were no dose-related increases in treatment-emergent adverse events or serious adverse events. Headache, nausea and sinusitis were the most common adverse events in patients receiving selonsertib.

3.2.2 GS-0976 (ACC inhibitor)

GS-0976 is an oral, investigational inhibitor of Acetyl-CoA carboxylase (ACC), one of several biologically relevant pathways associated with disease progression in NASH. ACC catalyzes the first step in hepatic de novo lipogenesis, the synthesis of fatty acids that contribute to hepatic steatosis and, subsequently, inflammation and liver fibrosis. Recent clinical trials have demonstrated that GS-0976 (20 mg taken orally once daily) when administered for 12 weeks was associated with statistically significant reductions in hepatic steatosis and a noninvasive marker of fibrosis (TIMP-1) compared to placebo. The study included 126 patients who were randomized to receive GS-0976 20 mg (n=49), GS-0976 5 mg (n=51), or placebo (n=26) once daily for 12 weeks. All patients in the study were diagnosed with NASH and liver fibrosis stages F1 through F3 based on biopsy, or by magnetic resonance elastography (MRE) and MRI proton density fat fraction (MRI-PDFF).

Patients receiving GS-0976 20 mg demonstrated significant decreases in liver fat content (measured by MRI-PDFF) compared to placebo after 12 weeks of treatment. Patients treated with GS-0976 20 mg also experienced a significant decrease in TIMP-1, a serum marker associated with liver fibrosis. Differences between GS-0976 5 mg and placebo were not statistically significant. Data for these efficacy endpoints are summarized in the table below.

Relative (%) Changes in Imaging, ALT and Serum Fibrosis Markers at Week 12*

Endpoint (Week 12)	GS-0976 20 mg (n=49)	GS-0976 5 mg (n=51)	Placebo (n=26)	P-values 20 mg vs. Placebo	P-values 5 mg vs. Placebo
MRI-PDFF	-28.9	-13.0	-8.4	**0.002**	0.142
≥30% reduction in MRI-PDFF, % (n/N)	48% (22/46)	23% (11/47)	15% (4/26)	**0.004**	0.433
MRE-stiffness	-5.5	-9.6	-12.5	0.100	0.743
Liver stiffness by FibroScan	-11.1	-8.4	-3.1	0.212	0.364
ALT	-20.5	-9.8	-6.7	0.176	0.765
TIMP-1	-7.9	-2.9	-1.5	**0.022**	0.301
PIII-NP	-13.9	-7.0	-0.5	0.107	0.605

* Unless indicated, all data are median relative (%) changes from baseline.

In other measures, including liver stiffness by FibroScan, liver stiffness by MRE, serum ALT and PIII-NP, a serum marker of fibrogenesis, no statistically significant differences were observed between the treatment and placebo arms of the study.

At week 12, a median relative change in triglycerides (TG) from baseline of +11 percent, +13 percent and -4 percent was observed in patients receiving GS-0976 20 mg, GS-0976 5 mg and placebo, respectively. Asymptomatic Grade 3 or 4 TG elevations (>500 mg/dL) were observed in 16 patients receiving GS-0976 20 mg (n=7) or 5 mg (n=9); the primary factor associated with such elevations was a baseline TG level >250 mg/dL ($p<0.001$). The majority of patients with such elevations either responded to fibrate or fish oil therapy (n=4) or resolved without additional treatment or cessation of GS-0976 (n=7). GS-0976 was well-tolerated. Nausea, abdominal pain and diarrhea were the most common adverse events.

3.2.3 GS-9674 (FXR inhibitor)

GS-9674 is a selective, non-steroidal agonist of the Farnesoid X receptor (FXR), a nuclear hormone receptor that is highly expressed in the gastrointestinal tract and liver.

FXR is the primary regulator of bile acid synthesis and plays important roles in glucose and lipid metabolism. Pre-clinical studies in animal models demonstrated that GS-9674 reduced hepatic steatosis and fibrosis, as well as serum levels of cholesterol, ALT and AST compared with untreated animals, and also had a dose-dependent anti-fibrotic effect, associated with lowering of portal pressure.

3.2.4 The Combination of Selonsertib with either GS-0976 or GS-9674

This combination has been evaluated in patients with advanced fibrosis due to nonalcoholic steatohepatitis (NASH) - the apoptosis signal-regulating kinase 1 (ASK1) inhibitor selonsertib with either the Acetyl-CoA carboxylase (ACC) inhibitor GS-0976 or the selective, non-steroidal Farnesoid X receptor (FXR) agonist GS-9674.

In this proof-of-concept study, 70 patients were treated with either selonsertib 18 mg plus GS-0976 20 mg (n=20), selonsertib 18 mg plus GS-9674 30 mg (n=20), or each monotherapy (n=10 per group) once daily for 12 weeks. All patients in the study were diagnosed with NASH and liver fibrosis stages F2 to F3 based on biopsy, or by magnetic resonance elastography (MRE) and MRI proton density fat fraction (MRI-PDFF). The greatest changes observed after 12 weeks of treatment in the study were decreases in liver fat content (measured by MRI-PDFF), which occurred in regimens containing GS-0976. Improvements in liver biochemistry and/or markers of fibrosis were also observed across both combination arms of the study compared to baseline. In patients treated with selonsertib plus GS-0976, kinetic labeling revealed the largest reduction in the fractional synthesis rate of lumican, a marker of fibrogenesis. Similar rates of adverse events were observed between patients treated with single-agent and combination therapies. No patient discontinued treatment prematurely.

3.3 Non-alcoholic Steatohepatitis (NASH)

3.3.1 Burden of Nonalcoholic Fatty Liver Disease (NAFLD) and Steatohepatitis (NASH)

NAFLD is the term encompassing all forms of fatty liver disease from benign steatosis to the necroinflammation and progressive fibrosis associated with NASH. The prevalence of NAFLD in North America ranges from 27 to 34% and the prevalence of NASH in the

United States ranges from 3 to 5% [52-54]. Similar prevalence has been described in studies from Europe and Asia.

Cirrhosis caused by NASH is an increasing indication for liver transplantation and is a common risk factor for hepatocellular carcinoma [55,56]. Patients with NASH are also at increased risk for cardiovascular disease, type 2 diabetes, and chronic kidney disease [55].

3.3.2 Clinical Presentation of NAFLD and NASH

Most individuals with NAFLD are typically asymptomatic and have normal liver blood tests. However, some individuals will have nonspecific symptoms or minimal elevations of liver enzymes; and, a minority will present with obvious clinical or laboratory features of advanced disease. Older age, hypertension, concomitant type 2 diabetes, obesity, dyslipidemia, and AST:ALT >1 are risk factors for NASH with fibrosis [52-55].

3.3.3 Diagnosis of NAFLD and NASH

NAFLD and NASH are suspected after exclusion of other causes of liver disease. The diagnosis of NAFLD is typically based on clinical, laboratory, and imaging criteria but the definitive diagnosis of NASH requires histological evaluation of a liver biopsy [45,57].

Given the poor patient acceptance and potential morbidity of liver biopsy a safer minimally-invasive alternative for NASH diagnosis is desirable. Clinical models, such as the NASH Clinical Scoring System or the NASH Predictive Index, incorporate demographic features and laboratory tests to generate a risk score for NASH [58,59].

Other minimally-invasive approaches include biomarkers, fibrosis biomarkers, liver stiffness measurements, and quantitative tests of liver function [60-65]. The HepQuant SHUNT test to be used in this study simultaneously measures clearance from portal and systemic circulations to detect changes in the portal circulation.

3.3.4 Measuring Disease Severity in NAFLD and NASH

Disease severity in NAFLD and NASH is based upon clinical, laboratory, and histologic criteria. Clinical and laboratory criteria are useful in defining disease severity only in the

late stages of disease. Histologic criteria define the spectrum from minimal change, through stages of advancing fibrosis and inflammation to cirrhosis [58,59].

In multi-variable analyses of clinical, laboratory, and histologic features, hepatic fibrosis (NASH CRN fibrosis stage >2) is the only factor that consistently predicts risk for liver-related mortality in NASH [57-65]. MELD (model for end-stage liver disease) and CTP (Child-Turcotte-Pugh) clinical models can define which cirrhotic NASH patients are at highest risk for death [65].

A non- or minimally-invasive method for measuring disease severity in NAFLD and NASH is desirable. The HepQuant SHUNT test to be used in this study provides a disease severity index (DSI) which correlates with other measures of disease severity. DSI can define baseline hepatic impairment and serial DSI measurements can track changes in response to treatments.

3.3.5 Treatment of NASH

There is currently no FDA-approved treatment for NASH. In the PIVENS trial of nondiabetic patients with NASH, Vitamin E, 800 IU/d x 96 weeks, reduced hepatic steatosis and inflammation but not hepatic fibrosis [59]. Clearly, new treatment options are needed for patients with NASH.

3.3.6 Pitfalls in Measuring Treatment Effect in NASH

A key pitfall in determining a positive (or negative) effect of any treatment of NASH is the lack of reliable minimally-invasive diagnostic tools. Generally, the efficacy of any intervention in NASH has been defined by histologic improvement on serial liver biopsies. However, poor tolerability and sampling error limit the accuracy and usefulness of liver biopsy as a monitoring tool.

Early identification of patients with a substantial improvement in hepatic function is of interest in the management of NASH. It is possible, even in relatively small trials, to link the results from an exploratory diagnostic tool, such as HepQuant SHUNT, to the histologic outcomes.

Standard blood tests, such as liver enzymes, bilirubin, albumin, and clotting times, can vary widely in concentration or activity due to a number of factors including non-hepatic

diseases, hemolysis, inflammation, diet, medications, gender, and age. These concerns make their use for monitoring treatment effects problematic.

Although liver biopsy is useful for establishing the diagnosis of NASH and can yield prognostic information it is invasive and risky [45]. Biopsy also suffers from sampling error, subjective scoring, and a potential for significant clinical morbidity.

Non-invasive elastography has virtually replaced liver biopsy in assessing disease severity in chronic hepatitis C but has not been validated in NASH. Elastography measures liver stiffness which is related to severity of liver fibrosis. However, the fat and inflammation which are the hallmark of NASH will likely alter the relationship of elastography to fibrosis and prognosis.

3.3.7 The HepQuant SHUNT Test in NASH

The HepQuant SHUNT test fulfills an unmet medical need because it is a minimally-invasive test of global liver function and physiology. With progression, liver diseases impair hepatocyte function and the portal circulation and these abnormalities become manifest as portal hypertension and portal-systemic shunting. The clinical consequences are coagulopathy, jaundice, varices, ascites and encephalopathy. HepQuant SHUNT quantifies the changes in liver function and the portal circulation from early on through later stages of disease.

Hepatic inflammation and hepatic fibrosis, which are hallmarks of NASH, impair hepatocyte function and hepatic perfusion. Most patients with NASH have underlying portal fibrosis which contributes to hepatic functional impairment via changes in the portal circulation. The progressive fibrosis and dysfunction ultimately leads to cirrhosis, portal hypertension, and portal-systemic shunting. Hepatocellular impairment, portal circulatory changes, portal hypertension, and portal-systemic shunting account for the major manifestations of NASH cirrhosis – jaundice, coagulopathy, fluid retention, varices and encephalopathy.

Underlying portal hypertension (PH) is a risk factor for poor outcome in NASH. PH can be measured by threading a catheter from the jugular vein into the portal vein to measure the hepatic venous pressure gradient (HVPG). Increased HVPG correlates with risk of cirrhosis, varices, ascites, and decompensation [27-29].

In NASH patients with PH, PH may reverse with resolution of the NASH. So why not use HVPG? First, HVPG is only effective as a monitoring tool in the patient with PH – many patients with NASH will not have PH and, accordingly, they will have normal HVPG which won't change with treatment. Second, HVPG is invasive, expensive, and requires specialized monitoring and specially trained professionals. As a result, HVPG can only be used selectively in specialized centers.

4.0 Preliminary Results

4.1 Measuring Disease Severity by the HepQuant SHUNT Test

4.1.1 Prior Studies

Over 1400 HepQuant SHUNT tests have been performed in over 650 persons. This experience encompasses healthy controls, and subjects with chronic hepatitis C, non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), primary sclerosing cholangitis (PSC), hepatocellular carcinoma (HCC), polycystic liver disease, and spans the spectrum from minimal to advanced fibrosis and compensated to decompensated disease [1-21]. In our prior studies of chronic hepatitis C, DSI correlated with ISHAK and METAVIR stages of fibrosis and predicted the likelihood of cirrhosis, varices, and risk for clinical outcome. DSI performed similarly in subjects with non-alcoholic fatty liver disease (NAFLD) and primary sclerosing cholangitis. Our data indicates that DSI scoring is effective in a wide range of liver diseases.

4.2 Measuring Treatment Effect by the HepQuant SHUNT Test

4.2.1 Prior Studies

Serial changes in DSI have been assessed in subjects with chronic hepatitis C, during and after antiviral treatment, and in subjects with PSC. There were two studies of HepQuant SHUNT measuring impact of sustained viral response (SVR), SOLAR-1 and HALT-C. In SOLAR-1, subjects were treated with ledipasvir/sofosbuvir/ribavirin, and in HALT-C, subjects were treated with peginterferon/ribavirin. HepQuant SHUNT was performed at baseline and at weeks 4, 24, 36, and 48 in SOLAR-1; and, at baseline and after approximately 2 years in HALT-C. In the PSC study, subjects underwent HepQuant SHUNT tests at baseline and after 1 year.

4.2.2 SOLAR-1 Clinical Trial

In SOLAR-1, DSI improved significantly by week 4. The improvement was greatest in transplant recipients with necro-inflammation and non-cirrhotic stages of fibrosis (ΔDSI -4.4±3.7, p<0.0001) and in transplant recipients with cirrhosis (ΔDSI -1.7±2.5, p<0.03). DSI did not change in non-transplant subjects with end-stage decompensated disease (ΔDSI 0.2±2.8, p=NS). Standard laboratory tests, MELD score, and CTP score failed to detect the improvement in liver function at week 4 [20].

The results in SOLAR-1 suggested that the decrease in DSI by week 4 was likely due to improvement in the hepatic microcirculation. First, ALT declined in parallel with the clearance of HCV RNA indicating reduction in necro-inflammation. Second, platelet count increased in LT Fibrosis and LT Cirrhosis, most probably from a reduction in splenic sequestration as hepatic resistance declined and portal hypertension improved. Third, the increase in platelet count correlated with the decrease in DSI. Fourth, although improvement in hepatocyte uptake of cholate might also account for the increases in HFRs and decline in DSI, there was no improvement in blood tests of hepatocyte function, such as bilirubin, albumin and INR. These findings are consistent with the interpretation that early hepatic improvement measured by drop in DSI reflects improvement in the hepatic microcirculation.

In CLD, necro-inflammation is a hallmark of the injury – baseline DSI will define the severity of the hepatic impairment at start of treatment. With successful treatment, necro-inflammation should reverse and be manifest as improved hepatic perfusion and hepatocyte uptake – events that should be detected by drop in DSI from the baseline test. If treatment has a very rapid onset of action, DSI could detect fibrosis reversal at the earliest time point after initiating treatment.

4.2.3 HALT-C and PSC Studies

The serial studies in HALT-C and PSC may not be as relevant to detection of early treatment effect since the follow-up DSI in these studies was either 1 year (PSC) or 2 years (HALT-C) after baseline testing. Nonetheless, the results in HALT-C demonstrated significant improvement in DSI after SVR and worsening of DSI in both untreated subjects and treated subjects who remained infected [4]. The study of PSC subjects identified 3 groups of PSC subjects: slow, moderate, and rapid progressors. The data in follow-up, one year later, confirmed slow progression in the slower progressor group

compared to the faster rate of progression in the moderate to rapid progressor groups [18].

5.0 Research Plan

5.1 Subjects

The subjects for the HepQuant study will be recruited from the patient cohorts for the three Gilead clinical trials with no more than N = 100:

- STELLAR 3: Adult subjects with NASH
NASH CRN Stage F3
- STELLAR 4: Adult subjects with NASH
NASH CRN Stage F4
Compensated Cirrhosis
- GS-US-454-4378 (NASH F3/F4) Adult subjects with NASH
NASH CRN Stage F3 or F4 (compensated)

5.2 Inclusion and Exclusion Criteria

To be enrolled in the HepQuant Study the subject must be co-enrolled in one of 3 Gilead trials noted in Section 5.1 and also:

- lack all exclusion criteria for the Gilead trial,
- not have had any previous dosing with Selonsertib, GS-0976 or GS-9674
- have adequate intravenous access for catheter placement and 6 blood draws,
- be able to drink the oral solution of 4D-cholate, and
- not have known hypersensitivity to albumin preparations, any ingredient in the formulation, or component of the HepQuant SHUNT Test Kit.

Subjects or persons with serious intercurrent medical or surgical illness, such as acute myocardial infarction, acute cerebral hemorrhage, sepsis, or other immediate life-threatening illness will be excluded. Subjects with extensive resection of large segments

of small intestine (short gut) or severe gastroparesis might not be able to absorb the oral dose of 4D-CA and are excluded.

Patients with cirrhotic stage of disease may not have had any clinical decompensating events (e.g., variceal hemorrhage, ascites, encephalopathy, spontaneous bacterial peritonitis).

5.3 Study Design

5.3.1 Dosing of Drugs

Dosing of drugs in the clinical trials is dictated by the Gilead clinical trial and is not further modified by the HepQuant Study. The dosing schedules are:

- STELLAR 3: Selonsertib 6 mg/d for 240 Wks, or
  Selonsertib 18 mg/d for 240 Wks, or
  Placebo
- STELLAR 4: Selonsertib 6 mg/d for 240 Wks, or
  Selonsertib 18 mg/d for 240 Wks, or
  Placebo
- GS-US-454-4378: Selonsertib 18 mg/d, or
  GS-0976 20 mg/d, or
  GS-9674 30 mg/d, or
  A combination thereof, or
  Placebo

The duration of treatment in the GS-US-454-4378 study is 48 weeks.

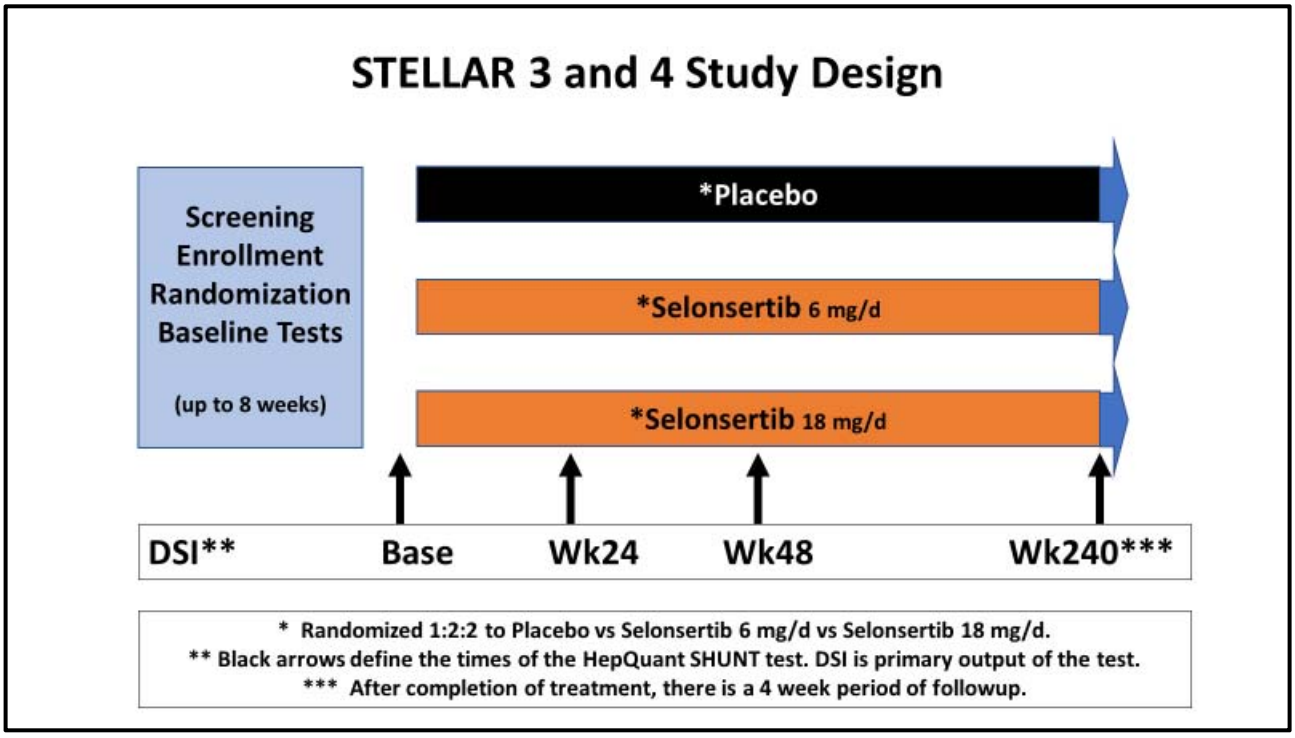
5.3.2 Timing of HepQuant SHUNT testing

The basic design for HepQuant SHUNT testing is similar across the clinical trials:

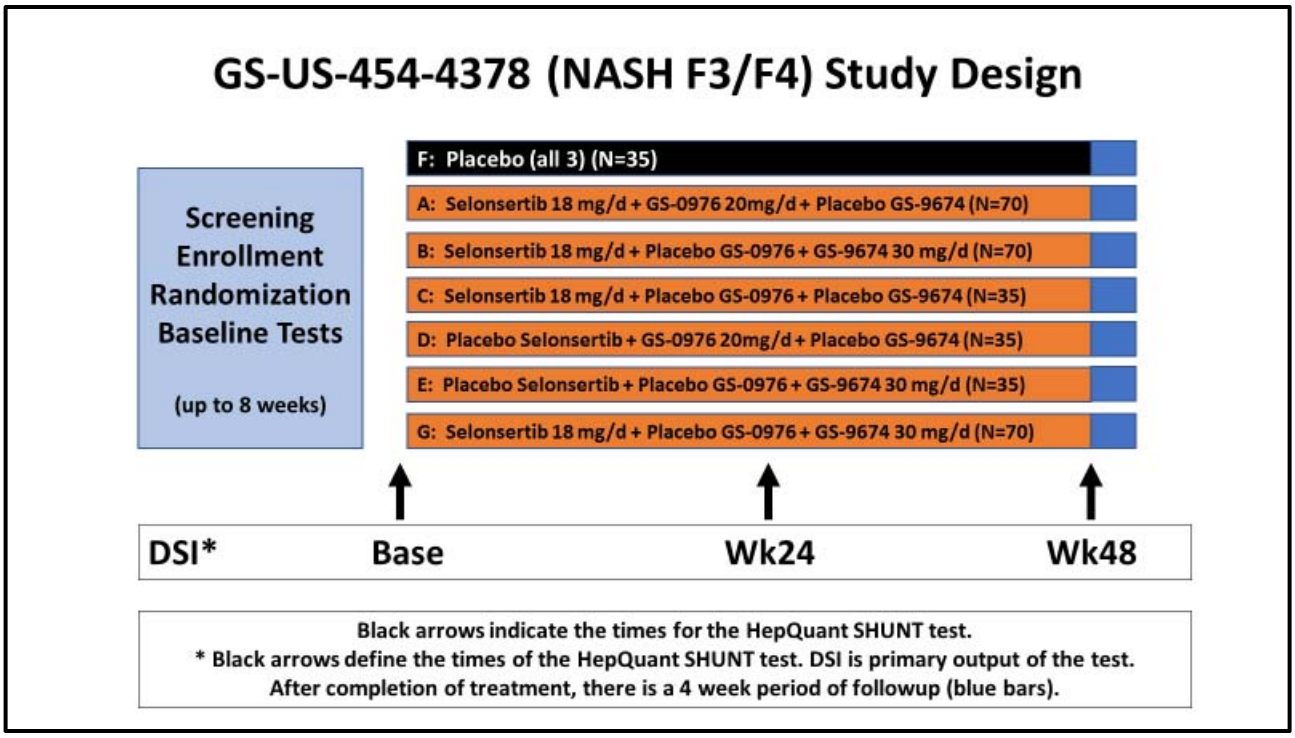
- In all trials, the HepQuant SHUNT Test is initially performed at baseline, prior to any treatment, to assess liver function and physiology at entry to the trial, time t_0.

- The HepQuant SHUNT Test is then performed serially to monitor the change in function and physiology, relative to baseline, during the placebo/treatment and post-treatment follow-up periods of the study. In all cases, the study visits for the HepQuant tests will coincide and align with the protocol-defined visit windows for the GILEAD clinical trials.

In the STELLAR 3 and STELLAR 4 trials, the HepQuant SHUNT test will be administered at baseline and Weeks 24, 48, and 240. The total length of time subject will be in the SHUNT study is approximately 252 weeks. The study design for the STELLAR studies is shown in the figure below.



In the GS-US-454-4378 (NASH F3/4) trial, the HepQuant SHUNT test will be administered at baseline and Weeks 24 and 48. The total length of time subject will be in SHUNT study is 52 weeks. The study design for the GS-US-454-4378 (NASH F3/4) trial is shown in the following figure.



The specific comparative data collected in the HepQuant study are given in Appendix C. The results of the HepQuant SHUNT test will be transferred to Gilead who will analyze the test results in comparison to other procedures and clinical outcomes recorded in the Gilead trials. HepQuant will query Gilead for the results of these analyses.

5.3.3 Treatment and Procedures

The HepQuant Study encompasses the administration of the HepQuant SHUNT test, use of the HepQuant SHUNT Liver Diagnostic Kit, and monitoring of the test and test outcome. The procedures, components, and outputs of the HepQuant SHUNT test have been previously summarized in Section 3.1 of this document.

All other aspects of the subject's care, treatments, management, and additional procedures are governed by the Gilead main clinical trial protocols. HepQuant will upload its test results to Gilead via secured portals and subsequently make requests of Gilead for specific analyses as defined via a collaborative research agreement between HepQuant and Gilead.

5.3.4 Administration of the HepQuant SHUNT test

Detailed instructions are provided in the Instructions for Use (Appendix B). The HepQuant SHUNT test is performed after an overnight fast or after at least 5 hours of fasting during the daytime. The morning dose of drug(s) is held until after completion of the HepQuant SHUNT test. The HepQuant SHUNT test requires venous access via a standard indwelling intravenous catheter, preferably placed in an antecubital vein. An oral solution of 4D-CA and an injectable solution of 13C-CA mixed with 25% human serum albumin are administered simultaneously over approximately 1 minute. If a problem is encountered with administering compounds simultaneously, the test is to be re-scheduled. If a rescheduled visit falls outside of the Gilead protocol-defined windows, sites should contact their study monitor for further instruction.

Blood samples are obtained at baseline and at 5±1, 20±2, 45±5, 60±5, and 90±5 minutes after cholate administration. The serum is separated and sent to the HepQuant laboratory for quantification of concentrations of endogenous cholate, 4D-CA and 13C-CA. Clearances are calculated from AUCs and dose and DSI and SHUNT are derived from the clearances.

5.3.5 Procedures by Study Visit (Also refer to Schedule of Events)

Week -8 to Day -1 (Screening)

- Informed Consent
- Review Inclusion/exclusion criteria
- Review continued eligibility
- Demographics
- Medical History
    - Hepatorenal Syndrome (Type I or Type II, treatment [if applicable])
    - Infection Related to Spontaneous Bacterial Peritonitis or SHUNT test or IV catheter (type, organism, treatment)
    - Hepatic Encephalopathy (treatment [if applicable], treatment type, whether controlled with treatment)
    - Ascites (treatment [if applicable], treatment type, whether controlled with treatment)
    - Variceal Hemorrhage (treatment [if applicable], treatment type)
- Physical Exam

Baseline/Day 1

- Informed Consent (if not performed Week -8 to Day -1/Screening)
- Review inclusion / exclusion criteria (if not performed Week -8 to Day -1/Screening)
- Review continued eligibility
- Demographics (if not performed Week -8 to Day -1/Screening)
- Medical history (if not performed Week -8 to Day -1/Screening)
    - Hepatorenal Syndrome (Type I or Type II, treatment [if applicable])
    - Infection Related to Spontaneous Bacterial Peritonitis or SHUNT test or IV catheter (type, organism, treatment)
    - Hepatic Encephalopathy (treatment [if applicable], treatment type, whether controlled with treatment)
    - Ascites (treatment [if applicable], treatment type, whether controlled with treatment)
    - Variceal Hemorrhage (treatment [if applicable], treatment type)
    - Varices and Endoscopy (whether performed, date, presence of varices, size of largest varix)
        - Any EGD results that were collected as part of the subject's standard of care. Window is ± 18 months of the SHUNT test date. These reports will be de-identified of any HIPAA information and sent to HepQuant with the samples.
- Physical exam (if not performed Week -8 to Day -1/Screening)
- Vital signs (respiratory rate, temperature, blood pressure, heart rate, weight, and height)
- Prothrombin time, INR (see table below)
- Hematology (see table below)
- Chemistry (see table below)
- **HepQuant SHUNT Test**
- Patient survey
- Adverse events

Week 24

- Review continued eligibility
- Medical History

  - Hepatorenal Syndrome (Type I or Type II, treatment [if applicable])
  - Infection Related to Spontaneous Bacterial Peritonitis or SHUNT test or IV catheter (type, organism, treatment)
  - Hepatic Encephalopathy (treatment [if applicable], treatment type, whether controlled with treatment)
  - Ascites (treatment [if applicable], treatment type, whether controlled with treatment)
  - Variceal Hemorrhage (treatment [if applicable], treatment type)
  - Varices and Endoscopy (whether performed, date, presence of varices, size of largest varix)
    - Any EGD results that were collected as part of the subject's standard of care. Window is ± 18 months of the SHUNT test date. These reports will be de-identified of any HIPAA information and sent to HepQuant with the samples.
- Vital signs (respiratory rate, temperature, blood pressure, heart rate, weight, and height) and laboratory tests
  - Prothrombin time, INR (see table below)
  - Hematology (see table below)
  - Chemistry (see table below)
- **HepQuant SHUNT Test**
- Patient survey
- Adverse events

Week 48

- Review continued eligibility
- Medical history
  - Hepatorenal Syndrome (Type I or Type II, treatment [if applicable])
  - Infection Related to Spontaneous Bacterial Peritonitis or SHUNT test or IV catheter (type, organism, treatment)
  - Hepatic Encephalopathy (treatment [if applicable], treatment type, whether controlled with treatment)
  - Ascites (treatment [if applicable], treatment type, whether controlled with treatment)
  - Variceal Hemorrhage (treatment [if applicable], treatment type)
  - Varices and Endoscopy (whether performed, date, presence of varices, size of largest varix)

        - Any EGD results that were collected as part of the subject's standard of care. Window is ± 18 months of the SHUNT test date. These reports will be de-identified of any HIPAA information and sent to HepQuant with the samples.
- Physical exam
- Vital signs (respiratory rate, temperature, blood pressure, heart rate, weight, and height)
- Prothrombin time, INR (see table below)
- Hematology (see table below)
- Chemistry (see table below)
- **HepQuant SHUNT Test**
- Patient survey
- Adverse events

Week 240 (Not applicable to GS-454-4378)

- Review continued eligibility
- Demographics
- Medical history
- Hepatorenal Syndrome (Type I or Type II, treatment [if applicable])
- Infection Related to Spontaneous Bacterial Peritonitis or SHUNT test or IV catheter (type, organism, treatment)
- Hepatic Encephalopathy (treatment [if applicable], treatment type, whether controlled with treatment)
- Ascites (treatment [if applicable], treatment type, whether controlled with treatment)
- Variceal Hemorrhage (treatment [if applicable], treatment type)
- Varices and Endoscopy (whether performed, date, presence of varices, size of largest varix)
    - Any EGD results that were collected as part of the subject's standard of care. Window is ± 18 months of the SHUNT test date, for the final study visit. These reports will be de-identified of any HIPAA information and sent to HepQuant with the samples.
- Physical exam
- Vital signs (respiratory rate, temperature, blood pressure, heart rate, weight, and height)

- Prothrombin time, INR (see table below)
- Hematology (see table below)
- Chemistry (see table below)
- **HepQuant SHUNT Test**
- Patient survey
- Adverse events

Local Laboratory Tests*		
Hematology	**Chemistry**	**Coagulation Profile**
WBC	Sodium	Prothrombin time
RBC	Potassium	INR
Hemoglobin	Calcium	
Platelets	Urea Nitrogen	
Neutrophils	Creatinine	
Lymphocytes	Albumin	
Monocytes	Bilirubin	
Eosinophils	Alanine Aminotransferase	
Basphils	Aspartate Aminotransferase	
Neutrophils (%)	Alkaline Phosphatase	
Lymphocytes (%)	Glucose	
	Chloride	

*To be collected prior to cholate/SHUNT test administration at each study visit

6.0 Descriptions, Risks, Justifications

6.1. Drug Safety Definitions

Abuse: Persistent or sporadic intentional excessive use of a medicinal product by a patient or clinical trial subject.

Adverse Event ("AE"): Any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have to have a causal relationship with this treatment. An Adverse Event (AE) can therefore be any unfavourable and/or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a

medicinal product, whether or not considered related to the medicinal product. AEs may also include pre- or post-treatment complications that occur as a result of protocol mandated procedures, lack of efficacy, overdose or drug Abuse/Misuse reports. Pre-existing events that increase in severity or change in nature during or as a consequence of participation in the clinical study shall also be considered AEs.

Adverse Reaction ("AR"): An untoward medical occurrence (unintended or noxious responses) considered causally related to an investigational or authorized medicinal product at any dose administered. Adverse Reactions may arise from Medication Errors, uses outside what is foreseen in the protocol or prescribing information (off-label use), Misuse and Abuse of the product, Overdose or Occupational Exposure where applicable.

Development Safety Update Report ("DSUR"): A report providing an annual review and evaluation of pertinent safety information collected during the reporting period to summarise the current understanding and management of identified and potential risks, describe new safety issues that could impact clinical trial subjects and provide an update on the status of the development program.

Medication Error: Any unintentional error in the prescribing, dispensing or administration of a medicinal product while the medication is in the control of a healthcare professional, patient or consumer.

Misuse: Use of a medicinal product that is intentional and inappropriate and not in accordance with its authorized product information.

Occupational Exposure: Exposure to a medicinal product as a result of one's professional or non-professional occupation.

Off-label Use: Where a medicinal product is intentionally prescribed by a Health Care Professional for a medical purpose not in accordance with the authorized product information with respect to indication, route, dose or patient population (e.g. the elderly). For avoidance of doubt, Off-Label Use will not apply in clinical trials.

Overdose: Administration of a quantity of a medicinal product given per administration or cumulatively which is above the maximum recommended dose as per the protocol or in the product labelling. The Parties agree that in the course of conducting a clinical study,

the terms of the clinical study protocol (as fully approved by all applicable bodies) overrides the local product labelling.

Pregnancy Reports: Reports of pregnancy following maternal or paternal exposure to the product.

Product Complaints: Complaints arising from potential deviations in the manufacture, packaging or distribution of the medicinal product.

Serious Adverse Event ("SAE") / Serious Adverse Reaction ("SAR"): An event or any untoward medical occurrence that at any dose either:

a) Results in death; or
b) Is life-threatening

The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe; or:

c) Requires in-patient hospitalisation or prolongation of existing hospitalisation; or
d) Results in persistent or significant disability/incapacity; or
e) Results in a congenital anomaly/birth defect; or
f) Results in a medically important event or reaction.

Explaining a medically important event: AEs requiring medical and scientific judgment to determine if expedited reporting is appropriate. Such events may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgement should be exercised in deciding whether an event is a medically important event. Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug Abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product shall be considered a medically important event and subject to expedited reporting requirements.

Special Situation Reports ("SSR"): One of a) Pregnancy, b) Abuse, c) Medication Error, d) Misuse, e) Off-Label Use, f) Overdose, g) Lack of Effect, h) AEs in infants following exposure from breastfeeding, i) AEs associated with Product Complaints or arising from Occupational Exposure. For the avoidance of doubt this applies to all reports including reports in a pediatric or elderly population.

Unexpected: An AE or AR where the nature and/or severity of the reaction is not consistent with the term or description used in the investigator brochure or product labeling.

NOTE: This is not an exhaustive list and any safety information will be reported to Gilead DSPH and appropriate regulatory bodies.

6.2 Test Administration Risks

BLOOD DRAWS
Drawing blood from a vein may cause local pain, bruising, occasional lightheadedness, fainting, and very rarely, infection at the site of the blood draw.

FASTING
Fasting could cause dizziness, headache, stomach discomfort, or fainting.

INTRAVENOUS CATHETER
In some cases, having an intravenous catheter inserted into your vein can cause infection or inflammation where it goes under the skin. In rare cases, it can cause a blood clot in the vein.

6.3 Cholates

6.3.1 Stable Isotope Labels (13C and 4D)

The labels used in the HepQuant test are cold and stable – they are NOT RADIOACTIVE and do not expose the individual to any radioactivity. No special monitoring is required. The HepQuant tests measure the clearance of 2 cholates labeled with molecular probes (carbon-13 (13C), and 4 deuteriums (4D)). Cholates are naturally occurring endogenous compounds found in the human body and 13C and D are also naturally occurring non-

radioactive cold stable isotopes. Cholates labeled with stable isotopes in the amounts administered in the HepQuant tests have no known harmful effects. Because clearance of cholate from the blood is a liver specific flow-dependent function, measuring cholate clearance is a minimally-invasive method for assessing the severity of liver disease in intact humans.

Cholates are naturally-occurring in the human body. The cholates in the HepQuant SHUNT test are modified by the addition of stable, cold (NON-RADIOACTIVE) isotopes. Neither naturally occurring nor the modified cholates used in the HepQuant SHUNT test have any known deleterious or adverse effects when given intravenously or orally in the doses used in HQ tests. The serum cholate concentrations that are achieved by either the intravenous or oral doses are similar to the serum concentrations of bile acids that occur after the ingestion of a fatty meal.

6.3.2 Manufacturing of Labeled Cholates

The cholate testing compounds will be purchased. The test compound 2,2,4,4-d4-cholate (d4-CA) has been previously studied under FDA Investigational New Drug (IND) application 65,123. The test compound 24-13C-cholate (13C-CA) has been previously studied under FDA IND 65,121. No adverse events occurred in over 1400 test administrations. These INDs are legacies to the current IDEs that govern use of the HepQuant SHUNT test in the Gilead trials. To date, the cholates used in this study have not been associated with any adverse events. However, they are still considered experimental and there may be unknown risks.

6.3.3 Formulations

Cholates are purchased in powder form and both powders are dissolved in sodium bicarbonate solution (USP grade) to achieve concentrations of 4 mg/mL and passed through 0.22 micron filters. The absolute concentrations of 4D-cholate in the 4D-cholate solution and the 13C-cholate in the 13C-cholate solution will be defined by LCMS methods – concentrations typically range from 3.5 to 4.2 mg/mL.

d4-CA aliquots of 10 mL (~40 mg) will be transferred to sterile glass vials and stored at ambient temperature. For 13C-CA aliquots of 5.5 mL (~22 mg) will be transferred to sterile glass vials and stored at ambient temperature. Sterility and absence of pyrogens will be confirmed prior to use.

A central pharmacy will formulate the test compounds, perform the sterile filtering, and prepare the sterile aliquots. Once concentration and sterility are confirmed the cholate solutions are transferred to a kit maker for assemblage of the HepQuant SHUNT Liver Diagnostic Kit.

NOTE: The vial containing the oral dose of 4D-CA contains the exact amount of 10 mL of 4D-CA (~40 mg). But, in contrast, the vial containing the intravenous dose of 13C-CA contains approximately 5.5 mL of 13C-CA (~22 mg) – an excess of 0.5 mL (2 mg) to allow for aspiration of exactly 5 mL into a syringe. Accurate testing requires administration of the entire 10 mL of 4D-CA (~40 mg) oral solution; and, exactly 5 mL of 13C-CA (~20 mg) intravenously.

6.3.4 Tolerability and Safety

Over 1400 HepQuant SHUNT tests have been performed in over 650 subjects without any untoward effects. In the GILEAD SOLAR-1 study of ledipasvir/sofosbuvir/ribavirin treatment of hepatitis C in decompensated cirrhosis or in liver transplant recipients, HepQuant SHUNT tests were performed 5 times over 48 weeks in each of 31 study participants. All 31 subjects completed a survey of their experience with the HepQuant SHUNT test after a mean (±SD) of 4.5±0.6 tests. The patient reported outcome survey is given in Appendix C. Pain was rated 0.5±0.8, where 0 was no pain and 10 was severe pain. Discomfort was rated 0.3±0.5, where 0 was no discomfort and 10 was severe discomfort. Inconvenience was rated 0.4±0.9, where 0 was no interference with daily activity and 10 was complete inability to conduct usual daily activity.

The recording of time commitment indicated <3 hours in 23 cases and >3 h in 8 cases. The overall experience with the test was rated 9.5±1.1, where 0 was very negative and 10 was very positive. Willingness to undergo additional testing was rated 9.1±1.8, where 0 was definitely not willing and 10 was very willing.

Ten subjects (32%) experienced 14 SAEs during the treatment course. The SAEs were 2 bouts of spontaneous bacterial peritonitis in the same patient, two admissions for anasarca in the same patient, and one each of dysphagia, myocardial infarction, biliary stricture, incarcerated umbilical hernia, nausea, hepatic encephalopathy, chest pain, diarrhea, anemia, and fever. None of the SAEs were attributed to HepQuant tests and only one, encephalopathy, was attributed to study drug (ribavirin).

6.3.5 Unknown / Unexpected Risks

The two cholates used in the HepQuant test for this study are labeled with stable (non-radioactive) forms of carbon and hydrogen that are found in nature and can be measured in blood. These forms of cholate have been registered with the FDA since 2002, and their use in humans has been monitored since that time. To date, the cholates used in this study have not been associated with any complaints or side effects. However, they are still considered experimental and there may be unknown risks.

The subject will be told of any new information that might cause him/her to change his/her mind about continuing to take part in this study.

6.3.6 Reporting

HepQuant is not collecting any information regarding adverse events, serious adverse events, and Special Situation Reports (SSR) with respect to the GILEAD products used in the GILEAD clinical trials. HepQuant is only monitoring the SHUNT test and procedure for AEs, SAEs, and Special Situations that are solely related to the HepQuant SHUNT test. HepQuant will inform study sites and GILEAD of AEs, SAEs, and SSRs that are documented to occur and are related to the HepQuant SHUNT test.

HepQuant will be responsible for the management of safety data from the Study and any associated regulatory reporting obligations for individual or periodic safety reports to the appropriate authorities and clinical investigators and applicable IRB/EC, in compliance with all applicable laws and the requirements of the IRB/EC.

The contact information for reporting of safety issues or adverse reactions is:

Andrea Herman
Manager, Clinical Accounts & Quality

U.S.A.

The contact information for reporting of safety issues, product concerns or feedback is:

Ring Central
800-793-8534
complaints@hepquant.com

6.4 Albumin

6.4.1 Use of Albumin in the HepQuant SHUNT Test

For the intravenous 13C-CA dose, exactly 5 mL of the 13C-CA intravenous solution are mixed with 5 ml of the albumin solution (25% w/v human serum albumin, Albutein ®-25 from Grifols Therapeutic, Inc, BLA 102478 – NOTE that the vial of albumin contains 20 mL; only 5 mL is added to the 13C-CA in the syringe, 15 mL is discarded). The 13C-CA/Albumin mixture is administered intravenously through the indwelling intravenous catheter over 1 minute by the nurse administering the test. The 4D-cholate/flavored solution is administered orally simultaneously.

6.4.2 Reported Reactions to Human Serum Albumin

6.4.2.1 Hypersensitivity

Albumin is given with the 13C cholate that is given intravenously. Albumin is a protein which is a normal part of your blood. In rare cases, hypersensitivity reactions to albumin have occurred. Some things that happen during any hypersensitivity reaction are:

- rash
- having a hard time breathing
- wheezing when you breathe
- sudden drop in blood pressure
- swelling around the mouth, throat, or eyes
- fast pulse
- sweating

- severe reactions are very rare (<0.01%; less than one chance in 10,000) but a severe reaction (called anaphylaxis) can lead to profoundly low blood pressure and even death.

Rare Hypersensitivity Reactions have been reported to human albumin preparations and include anaphylaxis or anaphylactoid reactions, fever, chills, rash, urticaria, pruritus, angioneurotic edema, and erythema or flushing.

6.4.2.2 Risk of Transmissible Agents

Because human albumin is prepared from pooled human plasma, there is a potential to pass human viruses (e.g., hepatitis viruses, HIV) and may carry a risk of transmitting Creutzfeldt-Jakob disease (CJD) or its variant CJD (vCJD). Through donor plasma screening and special procedures, like pasteurization to remove or inactivate any possible causes, reduce, but do not entirely eliminate the risk of transmission of disease causing agents. Nonetheless, the risk of transmission of viral disease with plasma-derived human albumin is considered extremely low. No cases of transmission of HBV, HCV, or HIV have been documented following use of commercially available human albumin. There are no documented cases of CJD or vCJD transmitted through plasma-derived preparations (including plasma-derived human albumin); the theoretical risk for disease transmission of CJD with commercially available human albumin is considered extremely low.

However, no purification method has been shown to be totally effective in removing the risk of viral infectivity from plasma-derived preparations and because new blood-borne viruses or other disease agents may emerge which may not be removed or inactivated by current manufacturing processes, the risks of human albumin are not entirely known.

6.5 Intravenous Catheter

Placing the indwelling intravenous catheter will cause minor pain and discomfort. With any blood draw there is a small risk of hematoma and a very small risk of a blood clot (1 in 100) or infection (1 in 1000). Over 1500 HepQuant SHUNT tests or the prototypical dual cholate research tests have been performed on over 600 individuals and most had the test multiple times. There has never been an adverse event - the risk from the test is

very small. A questionnaire will be completed by the subject at each of the testing periods to log their experience and tolerability of HQ tests (Appendix C).

6.6 Other

6.6.1 Data Security

Data Security: HepQuant study data will be collected and entered by the site staff into OmniComm TrialMaster Electronic Data Capture system. Additionally, HepQuant test and analytic data will be entered by HepQuant into EXCEL, ACCESS or REDCAP databases on HepQuant's HIPAA-compliant and encrypted servers that have been configured for 21CFR11 compliance.

HepQuant will transfer test results of the HQ shunt test to Gilead. The corresponding studies will be linked by de-identified study IDs.
The technology used for backing up the HepQuant file servers is Veeam. Backups run every 12 hours and are stored on a dedicated storage appliance. Onsite backups are retained for 90 days before expiring and being over written. All backups are stored with AES 256 encryption. Every night a backup copy is sent offsite to a secure datacenter in Arizona. Backups are retained in Arizona with the following schedules:

- 180 Daily backups
- 26 Full Weekly backups
- 24 Full Monthly Backups (First Sunday of the month)
- 16 Quarterly backups (First Sunday of the quarter)
- 5 Annual backups

Backup data replicated offsite is encrypted in transit and has hardware level encryption at rest.

6.6.2 Data Security Procedures

Storing and Securing Collected Data

- Chromatograms and associated peak areas are printed out during each LCMS analytical run and are considered the official raw data.

- Electronic copies (Agilent ChemStation software files) of the raw data from each run are stored on the local permanent disk storage (C drive) on the controlling computer attached to the LCMS system.

The ChemStation sequence used to direct the instrument is named for the day of the run (year as XX, month as XX, day as XX) and the analyst's initials. For example, the sequence of May 22, 2017, performed by SMH is named "170522 SMH" and the raw data generated from that sequence is automatically stored by ChemStation in a folder of that same name within the folder "C:\Chem32\1\data\". The sequence may be initiated to test system suitability or other parameters and only the first vial (system suitability standard) is injected and then the sequence is stopped if required (For example, the system suitability standard fails or the macro is not set up correctly). Each time the sequence is initiated a new subfolder is created. The subfolder that is created when the full sequence is completely executed is considered the run raw data set and at the completion of the run, it is renamed "XXXXXX" the date of the run. For example, the run Raw Data set would be "170522".

- During each run, a macro within Agilent Chemstation simultaneously generates in the folder "C:\Chem32\CORE\" a file called "rcvdata" containing a subset of the raw data (samples injection file #, injection time, vial #, sample name, injection #, peak retention time, peak area). After each run this raw data subset file is saved with a new name "rcvdata XXXXXX" with the same date as the raw data folder.

- For example, this file would be "rcvdata 170522". The "rcvdata XXXXXX" file is transferred to the analyst's encrypted laptop and used in the analysis to generate standard curves, QC results, and patient testing results.

- These data and folders, as well as all other laboratory data collection and analysis software on all the laboratory computers are only accessible when logged in by an authorized analyst. Each analyst is required to log in with a unique user name and unique password.

Backing Up Data

- After each run the raw data folder, "XXXXXX" is backed up by transferring it by the secure DSL directly from the C drive to the secure HepQuant server at RFC (Z drive). The raw data folders are also periodically backed up by transferring them to

read only DVDs that cannot be overwritten. These DVDs are then securely archived.

- After each run, the raw data subset file “rcvdata XXXXXX” is backed up by transferring it by the secure DSL directly from the C drive to the secure HepQuant server at RFC (Z drive).

- The secure server at RFC (Z drive) is itself backed up every night by CyberTrails to their secure server in Arizona.

Recovery of Data Backups

- When raw data backed up on the secure HepQuant server at RFC (Z drive) needs to be recovered, either for new analysis, supporting documentation, laboratory administration or integrity verification and validation, the raw data will be recovered from the secure HepQuant server at RFC (Z drive) via the secure DSL.

- Prior to transfer, the raw data folder, “XXXXXX”, on the Z drive will be saved with the new name “XXXXXX recover” and this folder will be transferred via the secure DSL to the laboratory LCMS computer folder which contains the original data.

- The reprocess function of Agilent Chemstation will then be activated on the file “XXXXXX recover” so that the macro generates a new raw data subset file and this file then will be saved with the name “rcvdata XXXXXX recover”.

Validation of Data Backups

- At intervals, dependent on project requirements, data backed up to the remote HepQuant server at RFC (Z drive) will be retrieved solely for the purpose of testing their completeness and integrity for validation of the data backup process.

- The SOP 001101 Form: “Laboratory Data Backup Validation Form” must also be completed by appropriate members of the validation team.

- On the form, paste screenshots of the file properties of the original raw data file folder and a recovered version of the raw data file folder and validate that the

recovered file folder is the same size as the original and contains the same number of files and subfolders.

- Open the “rcvdata XXXXXX recover” file and label every row of data as “recover” to distinguish it from the original data. Copy and paste the peak areas from the original “rcvdata XXXXXX” onto a new column and perform a test for equality [=IF (recover area = original area, TRUE, FALSE)] and check that all values return a result of TRUE to validate that peak areas of the file “rcv data XXXXXX recovery test” match original peak areas.

- Transfer of the “rcvdata XXXXXX recover” file will be to an analyst’s encrypted laptop and used it to generate standard curves and QC results. Validate that standard curves generated from the “rcvdata XXXXXX recover” match the original data standard curves in linearity (R2), slope, and back-calculated values. Validate that QC results values generated from the “rcvdata XXXXXX recover” match the original data QC results values.

6.6.3 Management of Abnormal Test Results

HepQuant will review all HepQuant SHUNT test results but will not distribute results to investigators, coordinators, GILEAD study personnel, primary care physicians, care providers, or subjects and no treatment or management decisions will be made on the basis of the HepQuant SHUNT results.

6.6.4 HepQuant Study Versus Gilead Clinical Trial

The HepQuant Study encompasses only the administration of the HepQuant SHUNT test, use of the HepQuant SHUNT Liver Diagnostic Kit, and monitoring of the test and test outcome. We do not anticipate any additional risks or issues related to the HepQuant SHUNT test other than those described above. AEs, SAEs, and SUSARs related to the HepQuant SHUNT test will be evaluated by HepQuant and reported by HepQuant to the appropriate regulatory authorities, including local IRBs and FDA and Gilead drug safety.

6.7 Pregnancy Protocol

Because cholates are naturally occurring with a pool size in humans of 1 to 5 g, the 20 and 40 mg doses of labeled cholates used in the HQ tests are unlikely to be harmful to a fetus. However, the effects of these compounds on the fetus are not definitively known.

Subjects will be co-enrolled in a placebo-controlled Gilead clinical trial of selonsertib. Subjects found to be pregnant during the Gilead clinical trial must discontinue study drug immediately. In this case, the subject must also stop participation in the HepQuant study.

It is recommended that all participants use a reliable form of birth control throughout the study. Double contraception using hormonal plus barrier methods is required. Hormonal birth control pills, intrauterine device (IUD), DepoProvera, Norplant, barrier methods (condom or diaphragm) plus a spermicidal agent, surgical sterilization, and complete abstinence are examples of reliable methods of birth control. Study participants will comply with the pregnancy precautions, and use one of the approved contraceptive methods, if applicable, as specified in the clinical protocols.

7.0 Potential Scientific Problems

7.1 Potential Interfering Substances in Laboratory Assay

7.1.1 Assay Performance

Use of concomitant medications could theoretically present potential interference in serum cholate measurement. The liquid chromatography-mass spectrometry (LCMS) technique used to measure cholate levels was validated according to FDA guidelines [66] for selectivity, accuracy, precision, recovery, stability, and freedom from interferences by serum components or medications. Freedom from interference was tested on blanks and at the LLOQ for each analyte with a number of metabolites and medications including: Bilirubin, Cholesterol, Carbamazepine, Oxazepam, Diazepam, Nordiazepam, Lorazepam, Temazepam, Flunitrazepam, Nitrazepam, Clonazepam, Alprazolam, Ephedrine, Codeine, Diphenhydramine, Nortriptyline, Propoxyphene, d-Amphetamine, d-Methamphetamine, Phenylpropanolamine, Phenmetrazine, Caffeine, Phencyclidine, Imipramine, Spironolactone, Furosemide, and dl-Propranolol. There was no interference at the

concentrations tested which were above those usually observed in patient serum samples.

7.2 Potential Complicating Medical Issues

7.2.1 Concomitant Medications

There are no known interactions of drugs or medications with the cholates used in the HepQuant SHUNT test. β-blockers or ACE inhibitors could affect the blood flow to the liver, so subjects who are currently taking either a beta blocker or an ACE inhibitor will be asked to delay taking their normal dose the morning of their testing. They can take the morning dose of these medications immediately after the 90 minute sample for the HepQuant SHUNT test is obtained. Delaying these medications could cause a temporary elevation in blood pressure but the risk would be minimal, similar to that of subjects who delay doses of medications in everyday life.

7.2.2 Associated Conditions

Subjects with serious intercurrent medical or surgical illness, such as acute myocardial infarction, acute cerebral hemorrhage, sepsis, or other immediate life-threatening illness will be excluded. Subjects with extensive resection of large segments of small intestine (short gut) or severe gastroparesis might not be able to absorb the oral dose of 4D-CA and are excluded.

7.3 Potential Interaction between Cholate and Study Drugs

Cholate, especially in the low doses used in the HepQuant SHUNT test, does not interfere with hepatic metabolism. Given these considerations there would be no suspected interaction between study drugs and cholate.

8.0 Data Analysis and Statistics

8.1 Sample Size Calculation

An important offshoot of our prior serial studies of the HepQuant SHUNT test was defining an average standard deviation for ΔDSI (average SD of ΔDSI = 3.5) that could

be used to determine sample sizes. The following table demonstrates the sample sizes required to detect a range of changes in DSI at 80% power or higher – for the case of the NASH trials. A sample size of 60 total subjects (N in following table), well below the size of our projected number of evaluable NASH patients could provide sufficient power to detect a difference in the ΔDSI of 2 to 3.

Actual Power	N1	N2	N	ΔDSI	σ	Alpha
0.80010	112	112	224	1.00	3.00	0.050
0.80084	199	199	398	1.00	4.00	0.050
0.80590	29	29	58	2.00	3.00	0.050
0.80590	51	51	102	2.00	4.00	0.050
0.82409	14	14	28	3.00	3.00	0.050
0.80486	23	23	46	3.00	4.00	0.050
0.81316	8	8	16	4.00	3.00	0.050
0.82409	14	14	28	4.00	4.00	0.050
0.85055	6	6	12	5.00	3.00	0.050
0.81383	9	9	18	5.00	4.00	0.050
0.80153	4	4	8	6.00	3.00	0.050
0.84086	7	7	14	6.00	4.00	0.050

NOTE: Once the treatment for the clinical trial is unblinded, if DSI is unchanged or worsens by 1 in the placebo arm, then one could detect as little as a 1 or 2 point reduction in DSI by selonsertib in the treatment arm.

8.2 Primary Objective

8.2.1 Outline of Approach

The primary objective is to determine the utility of the Disease Severity Index (DSI), derived from the HepQuant SHUNT test, for monitoring liver disease and treatment effects.

- Utility will be defined by measuring DSI and the change in DSI (ΔDSI) across all treatment arms.

- The clinical significance of a change in DSI will be evaluated by analyzing the relationships between changes in DSI and changes in histologic stage of disease, clinical models, and risk for clinical outcome.

Treatment effect will be determined after the completion of the clinical trial and after completion of Gilead's analysis of the results of the main clinical trial. Treatment effect will be defined from the difference in ΔDSI between treatment and placebo arms. Treatment effect defined by ΔDSI will also be compared to the treatment effect defined by changes in other tests and by clinical outcomes.

8.2.2 Comparators from the Gilead Clinical Trials

The primary clinical or histologic outcomes for the Gilead Clinical Trials are:

- STELLAR 3: Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from progression to cirrhosis
  Wk48: Freedom from decompensation, transplant, death
  Wk240: Decrease fibrosis stage ≥1; no worsening NASH
  Wk240: Freedom from progression to cirrhosis
  Wk240: Freedom from decompensation, transplant, death
- STELLAR 4: Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from decompensation, transplant, death
  Wk240: Decrease fibrosis stage ≥1; no worsening NASH
  Wk240: Freedom from decompensation, transplant, death
- GS-US-454-4378 (F3): Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from progression to cirrhosis
  Wk48: Freedom from decompensation, transplant, death
- GS-US-454-4378 (F4): Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from decompensation, transplant, death

The definition of worsening of NASH is a ≥1 point increase in hepatocellular ballooning or lobular inflammation using histologic criteria of the NASH CRN.

8.2.3 Statistical Considerations

Our Primary Objective is to determine the utility of the Disease Severity Index (DSI), derived from the HepQuant SHUNT test, to monitor liver disease and treatment effects. We propose a three-step analytical process to achieve this goal:

- First, without knowledge of treatment arm and for the group of subjects as a whole, we will track DSI and the change in DSI, ∆DSI, from baseline to on-treatment and follow-up timepoints.

- Second, we will apply separate survival models to evaluate ∆DSI as a continuous variable and a 2 point or more drop in DSI as dichotomous variable in the prediction of changes in other tests or models of liver disease severity and clinical outcomes.

- Third, after unblinding the study and completion of Gilead's analysis of the main clinical trial, we will make request of Gilead for allocation code to determine if there is a treatment effect, by comparing the difference between the ∆DSI of the treatment arm versus the ∆DSI of the placebo arm. The significance of this difference will be evaluated using two-sided t-tests and survival models.

Due to short enrollment period, only 5 STELLAR 3 and 4 STELLAR 4 subjects were enrolled into HQ-SHUNT-1701. STELLAR 3 and STELLAR 4 had two treatment arms (6 and 18 mg/d doses) and one placebo arm. Of the 9 subjects enrolled in HQ-SHUNT-1701, we would project that 6 would have been treated with selonsertib and 3 would have be taking placebo. Enrollment criteria, diagnosis, and F3/F4 stage of NASH is identical between the STELLAR studies and Gilead 454-4378. For the final analysis of treatment effects, once the code on all treatment arms from all studies is unblinded the projected 6 cases enrolled in HQ-SHUNT-1701 on selonsertib treatment from STELLAR studies will be added to the cases from Arm C of 454-4378 (selonsertib monotherapy) and the projected 3 cases enrolled in HQ-SHUNT-1701 on placebo from STELLAR studies will be added to the cases from Arm F (placebo) of the 454-4378 study.

Furthermore, we will link treatment effect defined by the change in DSI to effects of treatment on other tests and clinical outcomes (bleeding from varices or portal hypertensive gastropathy, ascites, encephalopathy, SBP, progression to cirrhosis, and

patient mortality or liver transplantation). In addition, we will use both generalized estimating equations (GEE) and profile analysis – profile of DSI between treatment and placebo groups at each time point – for additional primary endpoint analyses. The two NASH trials will be combined in the analysis of treatment effect.

8.3 Secondary Objective

8.3.1 Outline of Approach

The secondary objective will be to determine the ability of baseline DSI, performed prior to treatment, to assess the severity of liver disease by:

- correlation with other baseline tests of liver disease severity; and,
- prediction of risk for future clinical outcome.

8.3.2 Correlating baseline tests of liver disease severity

Our goal is to correlate the baseline DSI as a measure of liver disease severity with other measurements of liver disease severity.

The other measurements include:

- STELLAR 3: Liver histology, std labs, MELD & CP scores
- STELLAR 4: Liver histology, std labs, MELD & CP scores
- GS-US-454-4378: Liver histology, std labs, MELD & CP scores

Regression analysis will be used to obtain prediction and 95% CI of baseline NASH CRN fibrosis score, standard laboratory tests, and clinical models based on DSI. In a secondary analysis of the combined NASH trials, Gilead may use a discriminant analysis to compare the ability of baseline DSI to distinguish NASH F3 from NASH F4 to the ability of other tests and models to discriminate F3 from F4.

8.3.3 Baseline DSI as a predictor of Clinical Outcome

Our goal is to correlate the baseline DSI with risk for clinical outcomes. The primary clinical outcomes in the Gilead clinical trials include:

- STELLAR 3: Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from progression to cirrhosis
  Wk48: Freedom from decompensation, transplant, death
  Wk240: Decrease fibrosis stage ≥1; no worsening NASH
  Wk240: Freedom from progression to cirrhosis
  Wk240: Freedom from decompensation, transplant, death

- STELLAR 4: Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from decompensation, transplant, death
  Wk240: Decrease fibrosis stage ≥1; no worsening NASH
  Wk240: Freedom from decompensation, transplant, death
- GS-US-454-4378 (F3): Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from progression to cirrhosis
  Wk48: Freedom from decompensation, transplant, death

- GS-US-454-4378 (F4): Wk48: Decrease fibrosis stage ≥1; no worsening NASH
  Wk48: Freedom from decompensation, transplant, death

We will perform survival analysis for freedom from clinical outcome by Kaplan-Meier plots. Cox models will be used to estimate hazard ratios corresponding to 1 unit of DSI, controlling for other known risk factors of mortality (age, gender, smoking, etc). We will also apply separate survival models to evaluate DSI as a continuous variable and as above or below mean DSI as dichotomous variable in the prediction of clinical outcomes. In a secondary analysis of the combined NASH trials, we will use a discriminant analysis to compare the ability of baseline DSI to the ability of other tests and models to predict clinical outcomes.

9.0 Summary

This HepQuant study, conducted in parallel with three Gilead clinical trials, will determine the utility of DSI scores derived from the HepQuant SHUNT test for monitoring liver disease and treatment effects by measuring liver function and physiology. The results of DSI may be linked by Gilead to clinically meaningful changes in histology, laboratory tests, clinical models, and patient clinical outcomes. No more than 100 subjects will be

studied. The proposed HepQuant study and analytical plan will specifically measure the ability of DSI and ∆DSI to:

1. Define baseline disease severity, and
2. Detect clinically-meaningful changes in DSI by relating them to:
    a. changes in histology,
    b. changes in laboratory tests,
    c. changes in clinical models, and
    d. risk for clinical outcome.

The performance of DSI relative to other measurements made in these clinical trials could potentially establish DSI as an important tool for monitoring liver disease and treatment effects.

The HepQuant study will address key issues in NASH – is HepQuant SHUNT suitable for defining severity of the liver disease and monitoring the response of the liver to NASH treatment?

- The comparison of ΔDSI to change in NASH CRN fibrosis stage could provide evidence that supports use of ΔDSI as a potential surrogate for assessing changes in fibrosis stage – i.e., using ΔDSI as a minimally-invasive alternative to biopsy for monitoring treatment effects.

- Examining the baseline heterogeneity in functional impairment in NASH might identify high and low risk subgroups with different treatment response or clinical outcomes (such as progression to cirrhosis, or risk for clinical outcomes).

- Comparing the ∆DSI of the treatment arms to the ∆DSI of the placebo arm will demonstrate the effect of treatment. Not only baseline DSI but also ∆DSI will be correlated to the other tests, clinical models, and clinical outcomes. Changes in individual parameters of the HepQuant SHUNT test may identify the specific hepatic effects of the treatment.

Overall, the results from this study may support the use of HepQuant SHUNT as a tool or even endpoint for assessing efficacy of drug therapy of fibrotic stages of NASH in future trials.

10.0 References

1. Helmke S, Colmenero J, Everson GT. Noninvasive assessment of liver function. Curr Opin Gastroenterol. 2015 May;31(3):199-208.

2. Everson, GT, Martucci, MA, Shiffman, ML, Sterling, RK, Morgan, TR and Hoefs, JC. 2007. Portal-systemic shunting in subjects with fibrosis or cirrhosis due to chronic hepatitis c: The minimal model for measuring cholate clearances and shunt. Aliment Pharmacol Ther. 26: 401-410.

3. Everson, GT, Shiffman, ML, Morgan, TR, Hoefs, JC, Sterling, RK, Wagner, DA, Kulig, CC, Curto, TM and Wright, EC. 2008. The spectrum of hepatic functional impairment in compensated chronic hepatitis c: Results from the hepatitis c anti-viral long-term treatment against cirrhosis (HALT-C) trial. Aliment Pharmacol Ther. 27:798-809.

4. Everson, GT, Shiffman, ML, Hoefs, JC, Morgan, TR, Sterling, RK, Wagner, DA, Desanto, JL, Curto, TM and Wright, EC. 2009. Quantitative tests of liver function measure hepatic improvement after sustained virological response: Results from the hepatitis c anti-viral long-term treatment against cirrhosis (HALT-C) trial. Aliment Pharmacol Ther. 29: 589-601.

5. Everson, GT. 1993. Hepatic cysts in autosomal dominant polycystic kidney disease. Am J Kidney Dis. 22: 520-525.

6. Shrestha, R, McKinley, C, Showalter, R, Wilner, K, Marsano, L, Vivian, B and Everson, GT. 1997. Quantitative liver function tests define the functional severity of liver disease in early-stage cirrhosis. Liver Transpl Surg. 3: 166-173.

7. Everson, GT, Shiffman, ML, Hoefs JC, Morgan, TR, Sterling, RK, Wagner, D, Lauriski S, Curto, TM, Stoddard A, Wright, EC and The HALT-C Trial Group. 2012. Quantitative Liver Function Tests Improve the Prediction of Clinical Outcomes in Chronic Hepatitis C: Results from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis Trial. Hepatology 55:1019-1029.

8. Everson GT, Hoefs JC, Niemann CU, Olthoff KM, Dupuis R, Lauriski S, Herman A, Milne N, Gillespie BW, Goodrich NP, Everhart JE. Functional elements associated with hepatic regeneration in living donors after right hepatic lobectomy. Liver Transpl. 2013 Mar;19(3):292-304.

9. DeMark, BR, Everson, GT, Klein, PD, Showalter, RB and Kern, F, Jr. 1982. A method for the accurate measurement of isotope ratios of chenodeoxycholic and cholic acids in serum. J Lipid Res. 23: 204-210.

10. Kern, F, Jr., Everson, GT, DeMark, B, McKinley, C, Showalter, R, Braverman, DZ, Szczepanik-Van Leeuwen, P and Klein, PD. 1982. Biliary lipids, bile acids, and gallbladder function in the human female: Effects of contraceptive steroids. J Lab Clin Med. 99: 798-805.

11. Everson, GT. 1987. Steady-state kinetics of serum bile acids in healthy human subjects: Single and dual isotope techniques using stable isotopes and mass spectrometry. J Lipid Res. 28: 238-252.

12. Everson, GT, Fennessey, P and Kern, F, Jr. 1988. Contraceptive steroids alter the steady-state kinetics of bile acids. J Lipid Res. 29: 68-76.

13. Everson, GT, Daggy, BP, McKinley, C and Story, JA. 1992. Effects of psyllium hydrophilic mucilloid on ldl-cholesterol and bile acid synthesis in hypercholesterolemic men. J Lipid Res. 33: 1183-1192.

14. Lee, WM, Dienstag, JL, Lindsay, KL, Lok, AS, Bonkovsky, HL, Shiffman, ML, Everson, GT, Di Bisceglie, AM, Morgan, TR, Ghany, MG, Morishima, C, Wright, EC and Everhart, JE. 2004. Evolution of the HALT-C trial: Pegylated interferon as maintenance therapy for chronic hepatitis c in previous interferon nonresponders. Control Clin Trials. 25: 472-492.

15. Di Bisceglie, AM, Shiffman, ML, Everson, GT, Lindsay, KL, Everhart, JE, Wright, EC, Lee, WM, Lok, AS, Bonkovsky, HL, Morgan, TR, Ghany, MG, Morishima, C, Snow, KK and Dienstag, JL. 2008. Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. N Engl J Med. 359: 2429-2441.

16. Helmke SM, De Santo J, Herman A, et al. (2013) Dual Cholate Liver Function Testing is Better than Fibrosis Stage at Identifying Chronic HCV Patients with Medium/Large Varices and Predicting Future Clinical Outcomes. Hepatology 58: (S1)

17. Helmke SM, Wallack A, Herman A, Isberg H, Brocato M, Bhatt A, Lauriski S, Everson GT. Cholate Testing is Superior to MELD in Assessing Disease Severity in Patients with Primary Sclerosing Cholangitis. Liver Transplantation 2013; 19:S97-98.

18. Helmke SM, Wallack A, Herman A, Isberg H, Brocato M, Bhatt A, Lauriski S, Everson GT. Serial Cholate Testing is Better than ΔMELD in Assessing Disease Progression in Waiting List Patients with Primary Sclerosing Cholangitis. Liver Transplantation 2013; 19:S294.

19. Helmke SM, De Santo JL, Herman A, Lauriski S, Everson GT. (2013) A Disease Severity Index based on Dual Cholate Clearances and Shunt Outperforms Biopsy at Predicting Clinical Outcomes in Chronic Hepatitis C. Gastroenterology 144: (5):S951-952.

20. O'Leary JG, Burton JR, Helmke SM, et al. (2014) Early improvement in the HepQuant® (HQ)-SHUNT Function Test during Treatment with Ledispavir/Sofosbuvir in Liver Transplant Recipients with Allograft Fibrosis or Cirrhosis and Patients with Decompensated Cirrhosis who have not undergone Transplantation. Hepatology 60: (S1):1134A.

21. Steve M. Helmke, Jennifer DeSanto, Shannon Lauriski, Gregory T. Everson. A New Simple Quantitative Liver Function Test, HepQuant-STAT, Correlates with all Chronic HCV Ishak Fibrosis Stages, but Outperforms Fibrosis at Predicting the Risk of Future Clinical Outcomes. Hepatology (2015) 62(S1): 1111A (Abstract number 1849).

22. Stravitz RT, Reuben A, Mizrahi M, Lalazar G, Brown K, Gordon SC, Ilan Y, Sanyal A. Use of the methacetin breath test to classify the risk of cirrhotic complications and mortality in patients evaluated/listed for liver transplantation. J Hepatol 2015;63:1345-51.

23. Stravitz RT, Ilan Y. Potential use of metabolic breath tests to assess liver disease and prognosis: has the time arrived for routine use in the clinic? Liver International 2017;37:328-336.

24. Hermann-Georg Holzhutter, Johan Friso Lock, Pouria Taheri, Sascha Bulik, Andrean Goede, Martin Stockmann. Assessment of Hepatic Detoxification Activity: Proposal of an Improved Variant of the 13C-Methacetin Breath Test. PLoS ONE 8(8): e70780. doi:10.1371/journal.pone.0070780.

25. Stibbe KJ, Verveer C, Francke J, Hansen BE, Zondervan PE, Kuipers EJ, de Knegt RJ, van Vuuren AJ. Comparison of minimally-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients. Scand J Gastroenterol. 2011;46:962-72.

26. Iwakiri Y, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. J Hepatol 2014;61:912-24.

27. Samonakis, DN, Cholongitas, E, Thalheimer, U, Kalambokis, G, Quaglia, A, Triantos, CK, Mela, M, Manousou, P, Senzolo, M, Dhillon, AP, Patch, D and Burroughs, AK. 2007. Hepatic venous pressure gradient to assess fibrosis and its progression after liver transplantation for hcv cirrhosis. Liver Transpl. 13: 1305-1311.

28. Rincon, D, Ripoll, C, Lo Iacono, O, Salcedo, M, Catalina, MV, Alvarez, E, Nunez, O, Matilla, AM, Clemente, G and Banares, R. 2006. Antiviral therapy decreases hepatic venous pressure gradient in subjects with chronic hepatitis c and advanced fibrosis. Am J Gastroenterol. 101: 2269-2274.

29. Ripoll, C, Groszmann, R, Garcia-Tsao, G, Grace, N, Burroughs, A, Planas, R, Escorsell, A, Garcia-Pagan, JC, Makuch, R, Patch, D, Matloff, DS and Bosch, J. 2007. Hepatic venous pressure gradient predicts clinical decompensation in subjects with compensated cirrhosis. Gastroenterology. 133: 481-488.

30. Sandahl TD, Jepsen P, Thomsen KL, Vilstrup H. Incidence and mortality of alcoholic hepatitis in Denmark 1999–2008: a nationwide population based cohort study. J Hepatol 2011;54:760–764.

31. Liangpunsakul S. Clinical characteristics and mortality of hospitalized alcoholic hepatitis patients in the United States. J Clin Gastroenterol 2011;45:714–719

32. Lucey M, Mathurin P, Morgan TR. Alcoholic hepatitis. N Engl J Med 2009;360:2758–2769.

33. Cohen JA, Kaplan M. The SGOT/SGPT ratio – An indicator of alcoholic liver disease. Dig Dis Sci 1979;24:835–838.

34. Altamirano J, Fagundes C, Dominguez M, Garcia E, Michelena J, Cardenas A, et al. Acute kidney injury is an early predictor of mortality for patients with alcoholic hepatitis. Clin Gastroenterol Hepatol 2012;10:e3.

35. Kryger P, Schlichting P, Dietrichson O, Juhl E. The accuracy of the clinical diagnosis in acute hepatitis and alcoholic liver disease. Clinical versus morphological diagnosis. Scand J Gastroenterol 1983;18:691–696.

36. Mookerjee RP, Lackner C, Stauber R, Stadlbauer V, Deheragoda M, Aigelsreiter A, et al. The role of liver biopsy in the diagnosis and prognosis of patients with acute deterioration of alcoholic cirrhosis. J Hepatol 2011;55:1103–1111.

37. Hardy T, Wells C, Kendrick S, Hudson M, Day CP, Burt AD, et al. White cell count and platelet count associate with histological alcoholic hepatitis in jaundiced harmful drinkers. BMC Gastroenterol 2013;2013:55–59.

38. European Association for the Study of Liver. EASL clinical practical guidelines: management of alcoholic liver disease. J Hepatol 2012;57:399–420.

39. Altamirano J, Miquel R, Katoonizadeh A, Abraldes JG, Duarte-Rojo A, Louvet A, et al. A histologic scoring system for prognosis of patients with alcoholic hepatitis. Gastroenterology 2014;146:1231–1239.

40. Carithers Jr RL, Herlong H, Diehl AM, Shaw EW, Combes B, Fallon HJ, et al. Methylprednisolone therapy in patients with severe alcoholic hepatitis. A randomized multicenter trial. Ann Intern Med 1989;110:685–690.

41. Dominguez M, Rincon D, Abraldes JG, Miquel R, Colmenero J, Bellot P, et al. A new scoring system for prognostic stratification of patients with alcoholic hepatitis. Am J Gastroenterol 2008;103:2747–2756.

42. Dunn W, Jamil L, Brown LS, Wiesner RH, Kim WR, Menon KVN, et al. MELD accurately predicts mortality in patients with alcoholic hepatitis. Hepatology 2005;41:353–358.

43. Forrest EH, Evans C, Stewart S, Phillips M, Oo YH, McAvoy NC, et al. Analysis of factors predictive of mortality in alcoholic hepatitis and derivation and validation of the Glasgow alcoholic hepatitis score. Gut 2005;54:1174–1179.

44. Porter HP, Simon FR, Pope 2nd CE, et al. Corticosteroid therapy in severe alcoholic hepatitis. A double-blind drug trial. N Engl J Med 1971;284:1350–1355.

45. Rockey, DC, Caldwell, SH, Goodman, ZD, Nelson, RC and Smith, AD. 2009. Liver biopsy. Hepatology 49: 1017-1044.

46. Louvet A, Naveau S, Abdelnour M, Ramond MJ, Diaz E, Fartoux L, et al. The Lille model: a new tool for therapeutic strategy in patients with severe alcoholic hepatitis treated with steroids. Hepatology 2007;45:1348–1354.

47. Louvet A, Diaz E, Dharancy S, Coevoet H, Texier F, Thévenot T, et al. Early switch to pentoxifylline in patients with severe alcoholic hepatitis is inefficient in non-responders to corticosteroids. J Hepatol 2008;48:465–470.

48. Louvet A, Wartel F, Castel H, Dharancy S, Hollebecque A, Canva-Delcambre V, et al. Prospective screening of infection in patients with severe alcoholic hepatitis treated with steroids: early response to therapy is the key factor. Gastroenterology 2009;137:541–548.

49. Frebourg, T, Delpech, B, Bercoff, E, Senant, J, Bertrand, P, Deugnier, Y and Bourreille, J. Serum hyaluronate in liver diseases: Study by enzymoimmunological assay. Hepatology 1986;6:392-395.

50. Shaheen, AA, Wan, AF and Myers, RP. Fibrotest and fibroscan for the prediction of hepatitis c-related fibrosis: A systematic review of diagnostic test accuracy. Am J Gastroenterol 2007;102:2589-2600.

51. Tripodi, A, Caldwell, SH, Hoffman, M, Trotter, JF and Sanyal, AJ. Review article: The prothrombin time test as a measure of bleeding risk and prognosis in liver disease. Aliment Pharmacol Ther 2007;26: 141-148.

52. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD) and European Association for the Study of Obesity (EASO). EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol (2016), http://dx.doi.org/10.1016/j.jhep.2015.11.004.

53. Yousef Fazel, Aaron B. Koeniga, Mehmet Sayiner, Zachary D. Goodman, Zobair M. Younossi. Epidemiology and natural history of non-alcoholic fatty liver disease. Metabolism 2016;65:1017-1025.

54. Harrison S. NASH, From Diagnosis to Treatment: Where Do We Stand? Hepatology 2015;62:1652-1655.

55. Christopher D. Byrne, Giovanni Targher. NAFLD: A multisystem disease. J Hepatology 2015;62:S47–S64.

56. Heimbach JK, Watt KDS, Poterucha JJ, Ziller NF, Cecco SD, Charlton MR, Hay JE, Wiesner RH, Sanchez W, Rosen CB, Swain JM. Combined Liver Transplantation and Gastric Sleeve Resection for Patients With Medically Complicated Obesity and End-Stage Liver Disease. Am J Transplant 2013;13:363-368.

57. David E. Kleiner, Hala R. Makhlouf. Histology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in Adults and Children. Clin Liver Dis 2016;20:293–312.

58. Arun J. Sanyal, Elizabeth M. Brunt, David E. Kleiner, Kris V. Kowdley, Naga Chalasani, Joel E. Lavine, Vlad Ratziu, Arthur McCullough. Endpoints and Clinical Trial Design for Nonalcoholic Steatohepatitis. Hepatology 2011;54:344-353.

59. Arun J. Sanyal, Naga Chalasani, Kris V. Kowdley, Arthur McCullough, Anna Mae Diehl, Nathan M. Bass, Brent A. Neuschwander-Tetri, Joel E. Lavine, James Tonascia, Aynur Unalp, Mark Van Natta, Jeanne Clark, Elizabeth M. Brunt, David E. Kleiner, Jay H. Hoofnagle, Patricia R. Robuck, for the NASH CRN. Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis. N Engl J Med 2010;362:1675-85.

60. Christian Trautwein, Scott L. Friedman, Detlef Schuppan, Massimo Pinzani. Hepatic fibrosis: Concept to treatment. J Hepatology 2015;62:S15–S24.

61. Salvatore Petta, Marcello Maida, Fabio Salvatore Macaluso, Vito Di Marco, Calogero Camm, Daniela Cabibi, and Antonio Craxi. The Severity of Steatosis Influences Liver Stiffness Measurement in Patients With Nonalcoholic Fatty Liver Disease. Hepatology 2015;62:1101-1110.

62. Lai M. Is Liver Stiffness Measurement to Stage Fibrosis in Patients With Nonalcoholic Fatty Liver Disease Ready for Clinical Use? Hepatology 2015;62:997-998.

63. Parambir S. Dulai, Claude B. Sirlin, Rohit Loomba. MRI and MRE for minimally-invasive quantitative assessment of hepatic steatosis and fibrosis in NAFLD and NASH: Clinical trials to clinical practice. J Hepatology 2016;65:1006–1016.

64. Arun J. Sanyal, Colin Banas, Carol Sargeant, Velimir A. Luketic, Richard K. Sterling, Richard T. Stravitz, Mitchell L. Shiffman, Douglas Heuman, Adrian Coterrell, Robert A. Fisher, Melissa J. Contos, and Alan S. Mills. Similarities and Differences in Outcomes of Cirrhosis Due to Nonalcoholic Steatohepatitis and Hepatitis C. Hepatology 2006;43:682-689.

65. Goossens N, Hoshida Y, Song WM, Jung M, Morel P, Nakagawa S, Zhang B, Frossard J-L, Spahr L, Friedman SL, Negro F, Rubbia-Brandt L, Giostra E. Nonalcoholic Steatohepatitis Is Associated With Increased Mortality in Obese Patients Undergoing Bariatric Surgery. Clin Gastro Hep 2016;14:1619-1628.

66. Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May 2001.