CLINICAL STUDY PROTOCOL

Study Title:	A Phase 2, Double-Blind, Randomized Study Evaluating the Safety, Tolerability, and Efficacy of GS-4997 in Combination with Prednisolone versus Prednisolone Alone in Subjects with Severe Alcoholic Hepatitis (AH)	
Sponsor:	Gilead Sciences, Inc.	
IND Number:	129570	
EudraCT Number:	2016-000821-37	
Clinical Trials.gov Identifier:	TBD	
Indication:	Alcoholic Hepatitis (AH)	
Protocol ID:	GS-US-416-2124	
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Protocol Version/Date:	Original:	12 April 2016

CONFIDENTIALITY STATEMENT

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PROTOCOL SYNOPSIS

Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Study Title:	A Phase 2, Double-Blind, Randomized Study Evaluating the Safety, Tolerability, and Efficacy of GS-4997 in Combination with Prednisolone versus Prednisolone Alone in Subjects with Severe Alcoholic Hepatitis (AH)
IND Number:	129570
EudraCT Number:	2016-000821-37
Clinical Trials.gov Identifier:	TBD
Study Centers Planned:	Approximately 50 centers in the United States, Canada, France, Austria and United Kingdom
Number of Subjects Planned:	Up to 120 subjects will be randomized in order to obtain 100 subjects with severe AH
Target Population:	Males and non-pregnant, non-lactating females between 18-70 years of age with histologically-confirmed severe alcoholic hepatitis (Maddrey's Discriminant Function [Maddrey's DF] $\geq$ 32)
Duration of Study:	Participation in the study can last up to 26 weeks, which includes a 2-week screening period, a 4-week treatment period, and 20 week post treatment period.
Objectives:	The primary objective of this study is as follows: To evaluate the safety and tolerability of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe AH. The secondary objectives of this study are as follows: • To assess changes in hepatic synthetic function [liver biochemistry, Model for End-Stage Liver Disease [MELD] score, Child-Pugh score, the Lille model, Maddrey's Discriminant Function (Maddrey's DF), and [redacted] PPD [redacted]

- To assess mortality at 28 days, 12 weeks and 24 weeks;
- To determine the incidence of liver transplantation;
- To determine the incidence of hepatorenal syndrome (HRS);
- To determine the incidence of infection;
- To assess length of hospital stay.

The exploratory objectives of this study are:

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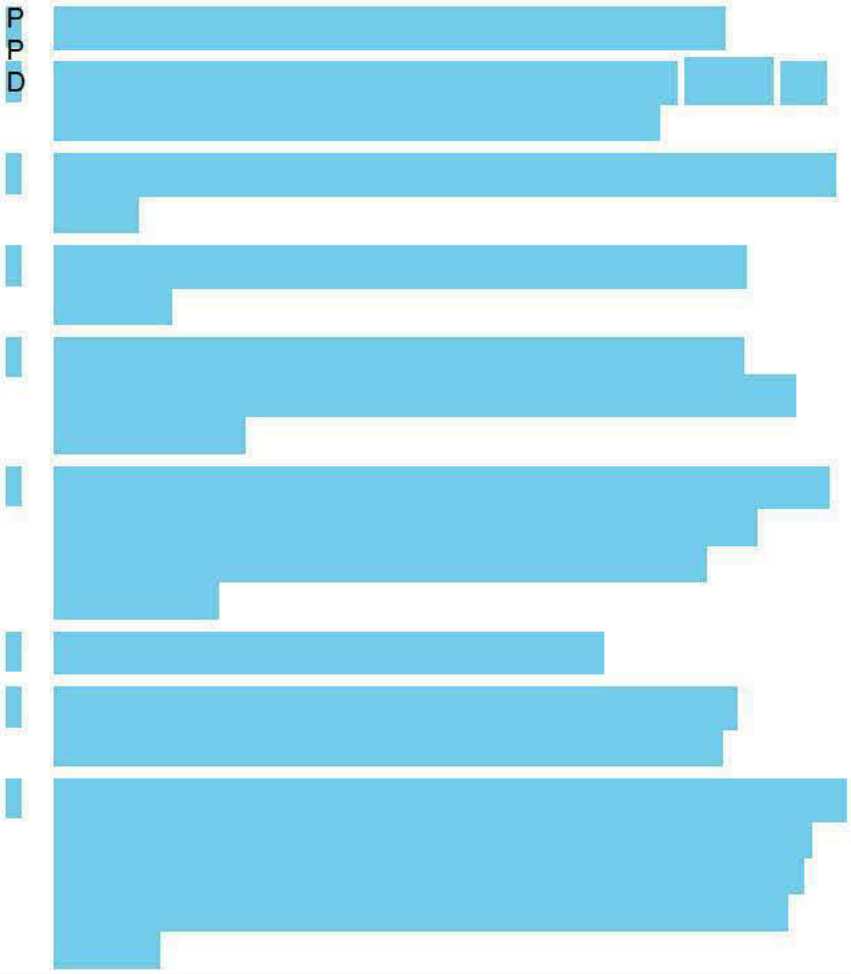

Study Design:

This is a Phase 2, double blind, proof-of-concept, randomized study evaluating the safety, tolerability, and biological activity of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe, histologically-confirmed AH.

Up to 120 subjects (for 100 evaluable AH subjects) will be randomized within strata (1:1) to either:

1. Group A (n = 50-60):

   GS-4997 18 mg once daily + prednisolone 40 mg once daily for 28 days

   OR

2. Group B (n = 50-60):

   GS-4997 placebo once daily + prednisolone 40 mg once daily for 28 days

Randomization will be stratified by MELD < or ≥25 at screening.

To allow for diagnoses inconsistent with severe AH in up to 20% of subjects based on liver biopsy findings, we will target randomization of up to 120 subjects into the study.

Diagnosis and Main Eligibility Criteria:

Inclusion Criteria:

1) Males and non-pregnant, non-lactating females between 18-70 years of age, inclusive based on the date of the screening visit;
2) Willing and able to give informed consent prior to any study specific procedures being performed. In subjects with hepatic encephalopathy (HE) which may impair decision-making, consent will be obtained per hospital procedures (eg, by Legally Authorized Representative);
3) Clinical diagnosis of severe AH based on:
   a) History of excessive alcohol consumption during the past 3 months (average of >40 g/d of alcohol for women and >50 g/d for men);
   b) Onset of jaundice within the past 3 months;
   c) Maddrey's DF ≥ 32 at screening;

4) All female subjects of childbearing potential must agree to use a highly effective method of contraception during intercourse from the screening visit throughout the study period and for 90 days following the last dose of study drug. If females utilize hormonal agents as one of their contraceptive methods, the same hormonal methods must have been used for at least 1 month before study dosing. Females on hormonal methods must also utilize a barrier method as another form of contraception as described in Appendix 3;
5) Male subjects must agree to use condoms during intercourse from screening through study completion and for 90 days following the last dose of study drug;
6) Male subjects must refrain from sperm donation from screening through at least 90 days following the last dose of study drug;
7) Female subjects must refrain from egg donation or harvest for 90 days after last dose of study drug;
8) Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, other study procedures, and study restrictions.

Exclusion Criteria:

1) Pregnant or lactating females;
2) Other causes of liver disease including chronic hepatitis B (hepatitis B surface antigen [HBsAg] positive), chronic hepatitis C (HCV RNA positive), acetaminophen hepatotoxicity, biliary obstruction, and autoimmune liver disease (subjects with fatty liver due to coexistent metabolic syndrome will not be excluded);
3) Serum aspartate aminotransferase (AST) >400 U/L or alanine aminotransferase (ALT) >300 U/L;
4) MELD >30 at screening;

5) Grade 4 Hepatic Encephalopathy (HE) by West Haven criteria;
6) Concomitant or previous history of hepatocellular carcinoma;
7) History of liver transplantation;
8) HIV Ab positive;
9) Uncontrolled sepsis;
10) Uncontrolled gastrointestinal (GI) bleeding or controlled GI bleeding within 7 days of screening that was associated with shock or required transfusion of more than 3 units of blood;
11) Type 1 hepatorenal syndrome (HRS) or renal failure defined as a serum creatinine >250 μmol/L (>2.8 mg/dL) or the requirement for renal replacement therapy;
12) Subjects dependent on inotropic (eg, epinephrine or norepinephrine) or ventilatory support (ie, endotracheal intubation or positive-pressure ventilation);
13) Portal vein thrombosis;
14) Acute pancreatitis;
15) Cessation of alcohol consumption for more than 2 months before baseline/Day1;
16) Severe associated disease (eg, cardiac failure, acute myocardial infarction, severe cardiac arrhythmias, severe pulmonary disease, neurologic disease) that may lead to premature mortality within the study period;
17) Malignancy within the 2 years prior to screening, with the exception of specific cancers that have been cured by surgical resection (eg, basal cell skin cancer). Subjects under evaluation for possible malignancy are not eligible;
18) Positive urine screen for amphetamines, cocaine or opiates (ie, heroin, morphine) at screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. Subjects with a positive urine drug screen due to prescription opioid-based medication are eligible if the prescription and diagnosis are reviewed and approved by the investigator;

19) Treatment with immunosuppressive drugs (eg, systemic corticosteroids, budesonide, tacrolimus, sirolimus, cyclosporine, azathioprine, mycophenolate mofetil, and methotrexate), pentoxifylline, or N-acetylcysteine (NAC) within 6 months of screening;

20) Use of the following CYP3A4 inhibitors (clarithromycin, conivaptan, grapefruit juice, itraconazole, ketoconazole, nefazodone, posaconazole, buprenorphine/naloxone, telithromycin, voriconazole) or CYP3A4 inducers (carbamazepine, phenytoin, rifampin, St. John's wort) within 2 weeks of baseline/Day 1;

21) Active ocular herpes simplex;

22) Any laboratory abnormality or condition that, in the investigator's opinion, could adversely affect the safety of the subject or impair the assessment of study results;

23) Participation in another investigational study of a drug or device within 1 month prior or within 5 half-lives of the prior investigational agent (whichever is longer) prior to screening;

24) Concurrent participation in another therapeutic clinical study;

25) Known hypersensitivity to the study drugs (GS-4997 and prednisolone), the metabolites, or formulation excipients;

26) Presence of any condition that could, in the opinion of the investigator, compromise the subject's ability to participate in the study, such as history of substance abuse other than alcohol use or a psychiatric or medical condition;

27) Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures.

Study Procedures/ Frequency:	Screening assessments should be completed within 14 days of Baseline/Day 1 visit. After the screening period and a randomization visit at baseline/Day 1, study visits will occur on Weeks 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24. At minimum, vital signs, physical examination (PE), Hepatic Encephalopathy (HE) and ascites assessment, safety laboratory tests, and review of adverse events, alcohol consumption, and concomitant medications will be done at every visit. PPD Screening assessments will include complete medical history, PE including vital signs, HE, ascites, Hepatorenal Syndrome (HRS) and infection assessment, laboratory assessments (hematology, chemistry, coagulation tests, HIV and hepatitis B and C serology; and urinalysis), pregnancy tests (for females of child-bearing potential), liver biopsy, liver ultrasound, standard 12-lead ECG, chest x-ray, AUDIT / SADQ questionnaires, adverse events and concomitant medication assessment. *Recent gastrointestinal bleeding:* Gastrointestinal bleeding will be treated and the subject stabilized during the screening period for at least 48 hours prior to randomization. *Sepsis:* All subjects will be screened for infection with chest radiography, urinalysis, urine culture, paracentesis with ascetic fluid (if clinically-detectable ascites is present) cell count, differential, and culture, and blood cultures. Sepsis will be defined as the presence of any positive culture or the requirement for initiation of antibiotics due to clinical or radiological signs of infection. Culture-negative pyrexia and leukocytosis on their own will not be regarded as signs of active sepsis, since these findings are often seen in subjects with AH without infection. Subjects with evidence of sepsis must be treated for a minimum of 2 days with appropriate antibiotics, and once sepsis is deemed under control by the investigator, the subject may continue screening and be randomized if eligible within the screening period.

Eligible subjects will be randomized to one of the two treatment groups. Prior to initial dosing, required baseline/Day 1 assessments will be performed and will include vital signs, PE, HE, ascites, HRS and infection assessment, laboratory assessments, pregnancy tests (for females of child-bearing potential), and review of adverse events, concomitant medication, CLDQ questionnaire and alcohol consumption.

PPD

While on study, subjects will undergo the following procedures and laboratory assessments:

- CLDQ questionnaire at baseline/Day 1, Weeks 4 and 24
- Sparse PK Sampling at baseline/Day 1 and at Weeks 1, 2 and 4
- Blood for Biomarkers at screening, baseline/Day 1, and at Weeks 1, 2, 4, 8, 12 and 24
- ELF™ Test and LOXL-2 at baseline/Day 1 and at Weeks 4, 12 and 24
- 12-lead ECGs at screening, baseline/Day 1, and at Weeks 4 and 8
- Fasting blood glucose, insulin and lipid profile at screening, baseline/Day 1 and at Weeks 4, 12 and 24
- Hemoglobin A1c at baseline/Day 1 and at Weeks 12, and 24
- Urine collection at screening
- Stool collection at baseline/Day 1 and at Weeks 4 and 24.

Intensive PK Sub-study

PPD

PPD

PPD

Test Product, Dose, and Mode of Administration:

- Treatment Group A: GS-4997 18 mg (1 x 18 mg tablet) AND prednisolone 40 mg (4 x 10 mg tablets) both administered orally once daily; n=50-60 subjects
- Treatment Group B: GS-4997 placebo (1 tablet) AND prednisolone 40 mg (4 x 10 mg tablets) administered orally once daily; n=50-60 subjects

Up to 60 subjects per arm will be randomized in order to obtain 50 subjects in each arm for the full analysis set (ie, subjects randomized with histologically-proven severe AH who took at least one dose of study drug)

Treatment will be administered for 28 days inclusive of inpatient, and if applicable, outpatient periods.

Reference Therapy: Prednisolone 10 mg tablets will be used.

Criteria for Evaluation:

Safety: Safety will be evaluated by examining the incidence of treatment emergent adverse events, including serious adverse events, clinical laboratory tests, ECGs, and vital signs. An independent, external Data Monitoring Committee (DMC) that consists of two hepatologists and a PhD statistician will convene prior to the study, once 10 subjects have been treated for 28 days and every 3 months thereafter to monitor the study for safety events including SAEs, deaths, grade 3 and 4 AEs, and grade 3 and above laboratory abnormalities.

Efficacy: The biological activity of prednisolone, and the combination of GS-4997 and prednisolone will be evaluated using clinical outcomes and biomarker variables.

Pharmacokinetics:	The plasma concentration of GS-4997 and its metabolite, GS-607509, will be determined. The plasma concentration of other GS-4997 metabolites and/or prednisolone may be explored. PK samples may also be used to measure protein-binding of GS-4997 and/or its metabolites. PPD

Statistical Methods:

Primary Endpoint:

The primary endpoint is the safety of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe AH.

Secondary Endpoints:

- Clinical outcomes
  - Mortality at 28 days, 12 weeks, and 24 weeks
  - HRS
  - Infection
  - Length of hospital stay
  - Liver transplantation
  - Measures of hepatic synthetic function:
    - Change from baseline in liver biochemistry: ALT, AST, GGT, albumin, alkaline phosphatase, bilirubin, and INR
    - Lille response (score <0.45) at Day 7, Lille score at Day 7 as a continuous variable, and combined score including Lille score at Day 7 and baseline MELD score
    - Change from baseline in prognostic indices: MELD, Child-Pugh score, Maddrey's DF
    - PPD

Exploratory Endpoints:

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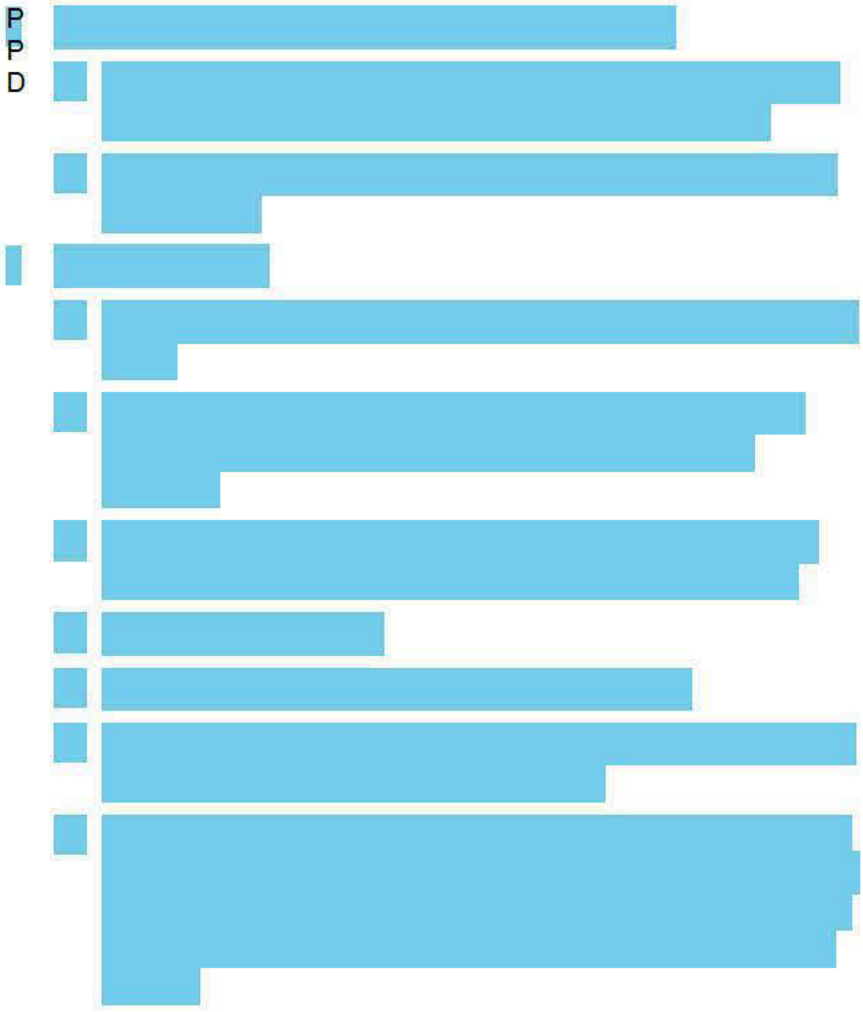
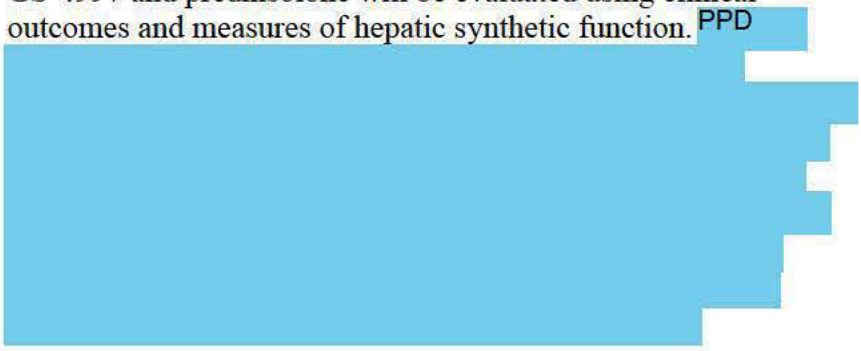


Efficacy Analysis:

The biological activity of prednisolone, and the combination of GS-4997 and prednisolone will be evaluated using clinical outcomes and measures of hepatic synthetic function. PPD



Exploratory Analysis:

PPD

Safety Analysis:

All safety data collected will be listed and be summarized, as appropriate, by treatment group. Safety endpoints will be analyzed by the number and percent of subjects with events or abnormalities for categorical variables or 8-number summary (n, mean, standard deviation, median, Q1, Q3, minimum, maximum) for continuous variables by treatment group.

Pharmacokinetic Analysis:

GS-4997 and GS-607509 plasma concentrations will be listed and summarized for all subjects. PPD

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Sample Size:

The number of subjects was chosen based on clinical experience with other similar proof-of-concept studies. PPD

This study will be conducted in accordance with the guidelines of Good Clinical Practice (GCP) including archiving of essential documents.

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

° C	degrees Celsius
° F	degrees Fahrenheit
ADR	adverse drug reaction
AE	adverse event
AH	Alcoholic Hepatitis
ALT	alanine aminotransferase
ARA	acid reducing agent
ASK1	apoptosis signal-regulating kinase 1
$ASK1^{-/-}$	apoptosis signal-regulating kinase 1 deficient
AST	aspartate aminotransferase
ATP	Adenosine triphosphate
AUC_{tau}	area under the plasma concentration versus time curve over the dosing interval (tau)
AUDIT	Alcohol Use Disorders Identification Test
β-hCG	beta human chorionic gonadotropin
BAP	Biomarker Analysis Plan
BMI	body mass index
BUN	blood urea nitrogen
CBC	complete blood count
cc	cubic centimeter
CI	confidence interval
CK18	cytokeratin (C18)
CL_{cr}	creatinine clearance
C_{last}	last observed quantifiable plasma/serum concentration of the drug
C_{max}	maximum observed plasma/serum concentration of drug
CLDQ	Chronic Liver Disease Questionnaire
CRO	contract (or clinical) research organization
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP3A4	cytochrome P4503A
DKD	Diabetic kidney disease
dL	Deciliter
DMC	Data Monitoring Committee
DSPH	Drug Safety and Public Health
DF	(Maddrey's) Discriminant Function
DP	drug product
EC	ethics committee
ECG	electrocardiogram

eCRF	electronic case report form
EDC	electronic data capture
eg	example
ELF™ Test	enhanced liver fibrosis test
EoT	End of Treatment
EU	European Union
FAS	Full analysis set
FDA	(United States) Food and Drug Administration
FIO_2	Fraction of Inspired Oxygen
FSH	Follicle-stimulating hormone
GGT	gamma glutamyl transferase
GI	gastrointestinal
GMP	Good Manufacturing Practice
GSI	Gilead Sciences, Inc.
HbA1c	Hemoglobin A1c
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular Carcinoma
hCG	human chorionic gonadotropin
Hct	Hematocrit
HCV	Hepatitis c virus
HDL	High-density lipoprotein
HDPE	High-density polyethylene
HE	hepatic encephalopathy
Hg	Hemoglobin
HIV	Human immunodeficiency virus
HLT	high-level term
HLGT	high-level group term
HOMA-IR	homeostatic assessment of insulin resistance
HRS	Hepatorenal Syndrome
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Identification
IEC	independent ethics committee
IEC	institutional ethics committee
IMP	Investigational Medicinal Product
IND	Investigational New Drug (Application)
INR	international normalized ratio

IRB	institutional review board
IUD	intrauterine device
IVD	In vitro diagnostic
IWRS	interactive web response system
kg	Kilogram
LOX	Lysyl oxidase
LOXL1	Lysyl oxidase-like 1
LOXL2	Lysyl oxidase-like 2
LOXL3	Lysyl oxidase-like 3
LOXL4	Lysyl oxidase-like 4
LPS	lipopolysaccharide
mAB	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
MELD	Model for End-Stage Liver Disease
µg	Microgram
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mm Hg	millimeters of mercury
NAC	N-acetylcysteine
NASH	Nonalcoholic steatohepatitis
PAH	Pulmonary arterial hypertension
PE	Physical Exam
PK	Pharmacokinetic
pH	measure of the acidity
PT	preferred term
PT	prothrombin time
PTM	Placebo to match
PTT	Partial prothrombin time
q	Every
QoL	Quality of Life
QT	electrocardiographic interval between the beginning of the Q wave and termination of the T wave, representing the time for both ventricular depolarization and repolarization to occur
RNA	ribonucleic acid
ROW	rest of world
SADQ	Severity of Alcohol Dependence Questionnaire
SADR	serious adverse drug reaction
SAE	serious adverse event

SAP	statistical analysis plan
SC	Subcutaneous
SOP	standard operating procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	An estimate of the terminal elimination half-life of the drug in serum/plasma/PBMC, calculated by dividing the natural log of 2 by the terminal elimination rate constant (λ_z)
TEAEs	Treatment emergent adverse events
T_{last}	last measured concentration
T_{max}	time (observed time point) of C_{max}
TQT	thorough QT
ULN	upper limit of the normal range
US	United States
wks	weeks

1. INTRODUCTION

1.1. Background

Alcoholic liver disease (ALD) encompasses a spectrum of disease ranging from reversible fatty liver to alcoholic hepatitis (AH) and cirrhosis. In the United States, ALD remains the second most common cause of liver cirrhosis after Hepatitis C virus (HCV) infection and is responsible for approximately 20% to 25% cases of liver cirrhosis {Gao et al 2011, Singal et al 2013}. In Western Europe, ALD is the most prevalent cause of advanced liver disease and cirrhosis is the leading cause of alcohol-related death among adults {European Association for the Study of the Liver (EASL) 2012}.

Alcoholic hepatitis represents one of the most serious forms of ALD and remains a significant cause of morbidity and mortality in North America and Europe. Approximately 60% of patients with ALD in the United Kingdom will have evidence of an alcohol-related hepatitis at presentation {Hislop et al 1983}. In the United States (US), the burden of AH is increasing, with an estimated 326,000 hospitalizations (0.8% of all admissions) in 2010, a 30% increase compared with 2002 {Jinjuvadia et al 2015}. The mean length of a hospital stay is 5 to 7 days with an average health care cost of $35,000 to $41,000. In patients with severe alcoholic hepatitis (typically defined by a Maddrey's Discriminant Function (DF) ≥ 32, short-term mortality rates are high (approximately 25% to 45%) {Akriviadis et al 2000, Mathurin et al 2011}.

Alcoholic hepatitis is a syndrome characterized by infiltration of the liver by inflammatory cells and hepatocellular injury. Several studies in rodent models and histopathological studies in humans suggest that lipopolysaccharide (LPS)-induced inflammation and oxidative stress are key drivers of hepatocyte death in AH {Chayanupatkul et al 2014, Lucey et al 2009, Meagher et al 1999}. Alcohol consumption increases intestinal permeability and bacterial translocation to the liver, resulting in LPS-induced hepatic inflammation. Subsequent hepatocellular injury occurs by neutrophil oxidative bursts, and FAS ligand and TNF-α induced hepatocyte apoptosis. In addition, ethanol strongly induces the expression of CYP2E1, which produces reactive oxygen species (ROS) as a by-product of ethanol metabolism, and reduces hepatic antioxidant capacity. Oxidative stress occurs as a result of these changes and directly induces hepatocyte mitochondrial dysfunction and necrosis and also sensitizes hepatocytes to apoptosis induced by TNF-α and FAS ligand {Cederbaum et al 2012}.

Pharmacologic agents that modulate these pathways have been used in the treatment of AH with limited success. Corticosteroid therapy which abrogates the inflammatory process is the current standard of care therapy for patients with severe AH. Compared with no treatment, a 28-day course of prednisolone improves 1-month mortality (approximately 35% vs. 20%), but shows limited impact on longer term outcomes {O'Shea et al 2010}. Indeed, 6-month mortality remains high despite prednisolone therapy {Mathurin et al 2011, Thursz et al 2015}. In addition to the limited efficacy of corticosteroids, there remains a high risk of infection in patients with severe AH who receive prednisolone {Louvet et al 2009}. In a cohort of 162 patients with AH (established by biopsy), 20% of patients had infections at hospital admission and 52% of those

treated with corticosteroids developed infections, including some patients with invasive aspergillosis {Michelena et al 2015}. In infected patients, the incidence of multiorgan failure (MOF, approximately 60%) and mortality (approximately 45% at 90 days) are substantially increased compared to uninfected patients (approximately 5% at 90 days). High-dose corticosteroids may also exacerbate glycemic control in diabetic patients and lead to an increased risk of gastrointestinal hemorrhage {Rudler et al 2015}. Other therapies that have been evaluated including pentoxifylline which modulates *TNF-α* transcription, N-acetylcysteine (an antioxidant), TNF inhibitors and antifibrotics, but none have shown convincing clinical benefit and/or are considered harmful {Mathurin et al 2011, Nguyen-Khac et al 2011, O'Shea et al 2005, Spahr et al 2002}. In light of the increasing burden of disease and limited therapeutic options, there remains a significant unmet medical need for alternative therapies with improved safety and efficacy for patients with severe AH.

1.2. GS-4997

1.2.1. General Information

GS-4997 is a small molecule that is a potent and selective inhibitor of apoptosis signal-regulating kinase 1 (ASK1). ASK1, a critical serine/threonine kinase, is an important signaling node through which oxidative stress promotes inflammation, proliferation, apoptosis, and fibrosis {Fujisawa et al 2007, Takeda et al 2008, Tobiume et al 2001, Watanabe et al 2005}. The ASK1 protein is ubiquitously expressed and is normally bound and repressed by thiol-containing antioxidant proteins in the cytosol and mitochondria {Fujino et al 2007, Takeda et al 2008, Zhang et al 2004}. In settings of elevated oxidative stress, ASK1 is autophosphorylated {Tobiume et al 2002}. The phosphorylated ASK1 then phosphorylates mitogen-activated protein kinase kinases (MAPKKs) 3, 4, 6, and 7, which in turn phosphorylate and activate the mitogen-activated protein kinases (MAPKs) p38 and c-Jun N-terminal kinase (JNK) {Nagai et al 2007, Takeda et al 2008}.

In pathological settings of oxidative stress, ASK1 is required for sustained activation of p38 and JNK, which promotes the expression of inflammatory cytokines (eg, interleukins 1β, 2, and 6), chemokines (eg, monocyte chemotactic protein 1, chemokine ligands 1 and 2), and matrix remodeling genes (ie, transforming growth factor beta, tissue inhibitor of metalloproteinase, and plasminogen activator inhibitor-1) {Matsuzawa et al 2005, Mnich et al 2010, Nakamura et al 2009, Takeda et al 2008, Terada et al 2007, Tobiume et al 2001, Yokoi et al 2006}. Activation of the ASK1 pathway also increases the expression of nicotinamide adenine dinucleotide phosphate-oxidase and induces mitochondrial damage leading to an increase in the production of reactive oxygen species (ROS), apoptosis,necrosis, and in certain contexts, insulin resistance {Adachi et al 2013, Gerczuk et al 2012, Imoto et al 2006, Nakagawa et al 2008, Takeda et al 2008, Toldo et al 2012, Watanabe et al 2005}.

A potential role for ASK1 in AH is supported by nonclinical studies demonstrating that oxidative stress, Fas ligand and TNF-α signaling activate ASK1 in the mouse liver {Nakagawa et al 2011}. In addition, *ASK1*$^{-/-}$ mice are resistant to acute liver injury caused by Fas activation, TNF-α (induced by LPS plus D-galactosamine), bile duct ligation, or acetaminophen overdose {Nakagawa et al 2011, Nakagawa et al 2008, Noguchi et al 2014}. Moreover, pharmacological inhibition of ASK1 in mice reduces hepatocellular injury and necrosis induced by acetaminophen

overdose, as well as cardiac and renal apoptosis and necrosis induced by acute ischemia/reperfusion injury {Toldo et al 2012, Xie et al 2015}. These effects correlated with reduced activation/phosphorylation of ASK1, p38, and JNK, and reduced mitochondrial dysfunction which could translate into improved liver function and resultant improved mortality in severe AH.

1.2.2. Preclinical Pharmacology and Toxicology

GS-4997 is a potent and selective ATP competitive inhibitor of the kinase ASK1 with an IC_{50} of 1.76 ± 0.85 nM. In an in vitro kinase selectivity screen (KINOMEscan, DiscoverRx) that evaluated the affinity of GS-4997 for the active sites of 451 kinases, GS-4997 had the highest affinity for ASK1 (K_d = 0.3 nM) followed by DYRK1A (K_d = 4.2 nM), and the N-terminal kinase domain of RSK4 (K_d = 8.6 nM). The K_d value of GS-4997 for ASK1 was 14-fold lower than that for DYRK1A, 29-fold lower than that for RSK4, and 50-fold lower than the K_d value determined for all other kinases.

The potency of GS-4997 to inhibit ASK1 activation and signaling was characterized in cellular assays and in vivo models of oxidative stress. In HK2 human renal proximal tubular epithelial cells, GS-4997 inhibited auranofin-induced ASK1 autophosphorylation and inhibited the downstream phosphorylations of p38 and JNK. GS-4997 efficacy was similar to results obtained when ASK1 expression was reduced by siRNA. The potency of GS-4997 to inhibit auranofin-induced p38 activation in human HK2 cells (EC_{50} 10.5 ± 1.3 nM) and human PBMCs (EC_{50} 10.8 ± 1.3 nM) were in-congruent with the potency of GS-4997 to inhibit ASK1 kinase in a biochemical assay (1.76 ± 0.85 nM). GS-4997 inhibited auranofin-induced production of the proinflammatory chemokine CXCL1 in human whole blood and in isolated human PBMCs with mean EC_{50} values of 131 ± 70 nM and 32 ± 13 nM, respectively. The approximate 4-fold difference in potency observed between whole blood and isolated PBMCs is likely due to plasma protein binding; the free fraction of GS-4997 in human plasma is 12%.

In safety pharmacology studies, GS-4997 had no effect on the central nervous and respiratory systems in male rats at doses up to 100 mg/kg, the highest dose tested. Mild to moderate prolongation of the QT interval corrected for heart rate (QTc; up to +17 msec) for up to 20 hours post dose and decreased systolic, diastolic and mean arterial pressure (up to -14%) were observed in cynomolgus monkeys after single dose of 75 and 10 mg/kg, respectively. The no observed effect level (NOEL) for cardiovascular effects was 3 mg/kg (human equivalent dose [HED] of 58 mg).

GS-607509, a major metabolite of GS-4997, has low potency to bind and inhibit ASK1 (approximately 100-fold less than GS-4997), low affinity for other off target kinases, and no significant binding to a broad panel of ion channels, transporters and receptors. In addition, the metabolite is > 99% protein bound in human blood. Therefore, GS-607509 is an inactive metabolite, which is not expected to have pharmacological activity at clinically relevant exposures.

1.2.3. Preclinical Drug Metabolism and Pharmacokinetics

Consistent with the moderate to high bioavailability seen in nonclinical species, GS-4997 shows high, concentration-independent forward permeability across Caco-2 monolayers, with low efflux. GS-4997 has a moderate volume of distribution, close to that of total body water. Systemic clearance in nonclinical species is well predicted from the rates of metabolism by hepatic microsomal fractions and hepatocytes. GS-4997 has good metabolic stability with human hepatic material in vitro and low clearance in vivo. In vitro, the main route of metabolism of GS-4997 involves N-dealkylation, resulting in loss of the isopropyl group (metabolite M33, GS-607509. In rat, other routes of metabolism were detected, including oxidation (with subsequent glucuronidation) and hydrolysis of the amide. GS-4997 is unlikely to cause clinical interactions through inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19 or CYP2D6. Substrate dependent inhibition was observed for CYP3A (IC_{50} 2.69 μM). The potential for the metabolite GS-607509 to cause inhibition was also investigated, and it had no inhibitory effect on the activities of human hepatic microsomal CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A (IC_{50} > 25 μM); thus, it is unlikely to affect the PK of drugs metabolized by these enzymes.

The effects of GS-4997 and GS-607509 on the activity of the conjugating enzyme, uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), were also determined. GS-4997 inhibited this enzyme with an IC_{50} of 1.9 μM, while GS-607509 inhibited this enzyme to a lesser extent (IC_{50} 19.4 μM). No clinically relevant or dose-dependent increases in bilirubin levels were noted in the first-in-human Phase 1 study with GS-4997 (GS-US-223-0102). As such, GS-4997 and GS-607509 are unlikely to cause clinically relevant inhibition of UGT1A1.

GS-4997 was a weak inhibitor of the breast cancer resistance protein (BCRP), the organic anion transporter proteins (OATP)1B1, and OATP1B3 (IC_{50} for each ≥ 9.9 μM). GS-4997 is a more potent inhibitor of P-gp, organic cation transporters (OCT1 and OCT2), and multidrug and toxin extrusion 1 (MATE1) transporter (IC_{50} 3.4, 2.8, 5.5, and 2.4 μM, respectively). GS-607509 was a weak inhibitor of all transporters (IC_{50} > 10 μM) except MATE1 (IC_{50} 6.4 μM). As such, the overall potential for interactions for GS-4997/metabolite is low for most transporters. GS-4997 and GS-607509 are not expected to be a clinically relevant inducer through activation of either AhR or PXR.

Elimination of GS-4997 and its metabolites is likely to occur through a mixture of urinary, biliary and intestinal excretion.

1.2.4. Preclinical Toxicology

The toxicity profile of GS-4997 has been assessed in rats and cynomolgus monkeys administered GS-4997 orally for up to 26 weeks in rats or 39 weeks in monkeys. Additionally, studies were conducted to assess the effect of GS-4997 on embryofetal development as well as the potential for genotoxicity and phototoxicity. Target organs/tissues identified in the repeat-dose toxicity studies with GS-4997 were the GI (monkey only; profuse diarrhea), kidney (monkey - tubule degeneration/necrosis/regeneration; rat - tubular basophilia, eosinophilic droplets and pigment deposition), hematopoietic tissue (mild decrease red blood cell parameters), lymphoid tissue

(monkey only; decreased lymphocytes), adrenal (rat only; cortical hypertrophy), and lung (monkey only; phospholipidosis). In general, findings were reversible, monitorable, and only occurred at doses that exceeded the projected exposure (AUC_{24h}) at the proposed maximum human dose by at least 5-fold. In addition, embryo-fetal effects were observed in association with maternal toxicity in rats and rabbits at exposures (AUC_{24h}) that were 67- and 12-fold higher, respectively. The no-observed-adverse-effect level (NOAEL) from the long term repeat dose toxicity studies in rat and monkey were 15 and 5 mg/kg/day, respectively. These respective doses were associated with exposures (AUC_{24h}) that were 19- and 1-fold the projected steady state exposure in humans at the maximum proposed human dose of 18 mg once daily. Furthermore, GS-4997 was nongenotoxic and nonphototoxic.

For further information on GS-4997, refer to the investigator's brochure (IB) for GS-4997, 3rd edition, dated 13 May 2015.

1.2.5. Clinical Trials of GS-4997

As of 08 February 2016, four Phase 1 studies with GS-4997 have completed and two Phase 1 studies with GS-4997 are ongoing. In the completed and ongoing Phase 1 studies, 253 healthy subjects have been dosed with GS-4997.

Three Phase 2 studies of GS-4997 in subjects with diabetic kidney disease (DKD) (GS-US-223-1015), pulmonary arterial hypertension (PAH) (GS-US-357-1394), and nonalcoholic steatohepatitis (NASH) (GS-US-384-1497) are ongoing.

1.2.5.1. An Adaptive, Single and Multiple Dose Phase 1 Study of GS 4997 in Healthy Volunteers to Evaluate the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and its Drug-Drug Interaction Potential Using a Probe CYP3A Substrate (Study GS-US-223-0102)

Single- and multiple-dose oral administration of GS-4997 once daily over 14 days was well tolerated in healthy adult subjects. GS-4997 exposure increased in an approximately dose-proportional manner over the dose-range tested (1 to 100 mg) following single and multiple oral administrations (once daily for 14 days) under fasted conditions. GS-4997 terminal $t_{1/2}$ values ranged from 15 to 23 hours and T_{max} ranged from 2 to 4 hours following single and multiple dosing. GS-607509 is a major, inactive metabolite of GS-4997 that, upon once daily dosing of GS-4997, accumulated to approximately 6-fold of GS-4997 AUC. Urinary excretion appears to be a minor pathway of elimination of GS-4997 and GS-607509. No food effect on the PK of GS-4997 was demonstrated. As such, GS-4997 may be administered with or without food. Changes in midazolam PK upon co-administration with GS-4997 were minimal and, therefore, GS-4997 is not considered a clinically relevant CYP3A inhibitor. No clinically significant vital signs changes, ECG changes, or laboratory changes were noted on study. These results provide adequate characterization of the PK and safety of GS-4997 to enable further clinical investigation with this molecule. Further information on this study is available in the Investigator's Brochure for GS-4997.

After completion of this study, a concentration-QT (C-QT) analysis of intensive, time-matched ECG data collected in this Phase 1 study was conducted as an alternative to a dedicated thorough QT (TQT) study to evaluate changes in QT interval with GS-4997. The primary C-QTc analysis evaluated the effect of GS-4997 and its metabolite (GS-607509) on time-matched, baseline-adjusted, placebo-corrected QTcF (ΔΔQTcF) intervals. Categorical analysis and C-QTc modeling demonstrated that there were no clinically relevant relationships between ΔΔQTcF and GS-4997 dose or plasma concentrations of GS-4997 and its metabolite (GS-607509). Thus, at exposures achieved at the proposed dose range (up to 18 mg), this analysis provided adequate evidence to exclude a clinically relevant effect on QTc interval. Based on these results, a waiver for conducting a TQT study was granted by the Division of Cardiovascular and Renal Products at the Food and Drug Administration.

1.2.5.2. A Phase 1 Study to Evaluate the Effect of Acid Reducing Agents on GS-4997 Pharmacokinetics (GS-US-223-1432)

This Phase 1, open-label, single-center 2-cohort study was designed to evaluate the safety, tolerability, and PK of GS-4997 when administered alone and in combination with gastric acid reducing agents (famotidine, omeprazole) in healthy adult males and females of nonchildbearing potential. Cohort 1 investigated the potential interaction of a H_2-receptor antagonist (famotidine [Pepcid®]) when coadministered with GS-4997 (20 mg). Cohort 2 investigated the potential interaction of a proton pump inhibitor (omeprazole [Prilosec®]) when coadministered with GS-4997 (20 mg).

No clinically relevant drug-drug interactions occurred between GS-4997 and famotidine or omeprazole. As such, GS-4997 may be administered with gastric acid reducing agents. No new safety concerns for GS-4997 in healthy subjects were identified, either when administered alone as a single 20-mg oral dose or when coadministered with famotidine (40 mg) orally twice daily or omeprazole (20 mg) orally once daily.

1.2.5.3. A Phase 1 Study to Evaluate the Effect of GS-4997 on a Probe Substrate of Drug Transporter P-glycoprotein (P-gp) and the Effect of Inhibitors/Inducers of Cytochrome P450 3A (CYP3A) and Inhibitors of Organic Anion Transporting Polypeptide (OATP) 1B1 and 1B3 on GS-4997 Pharmacokinetics (GS-US-223-1434)

This Phase 1 open-label, single-center, multiple-cohort study was designed to evaluate the safety of GS-4997 and the effect of GS-4997 on the drug transporter P-gp and the effect of inhibitors/inducers of CYP3A and inhibitors of OATP1B1/1B3 on the PK of GS-4997 in healthy adult males and females of nonchildbearing potential administered GS-4997 alone or in combination with various probe drugs.

GS-4997 is a very weak P-gp inhibitor and may increase exposures of sensitive P-gp substrates such as digoxin. GS-4997 is not a sensitive substrate of hepatic uptake transporters OATP1B1/1B3 and may be coadministered with OATP1B1/1B3 inhibitors. Because rifampin significantly decreased GS-4997 plasma exposure, exclusion of strong CYP3A4 inducers in combination with GS-4997 in long-term clinical studies should be considered. While multiple

doses of ritonavir, a strong CYP3A4 inhibitor, significantly increased GS-4997 plasma exposure, and bosentan, a moderate CYP3A4 inducer, decreased GS-4997 plasma exposure, the clinical relevance of these findings has not been established. A 2-fold increase in bosentan plasma concentrations has been observed with bosentan and ketoconazole coadministration, which did not warrant dose adjustment {Actelion Pharmaceuticals LTD 2012}. Thus, the lower increase in bosentan exposure observed with GS-4997 does not warrant dose adjustment in long-term clinical studies. Single or multiple oral doses of GS-4997 (20 mg) administered alone or in combination with oral doses of digoxin (0.5 mg, once daily), rifampin (600 mg, once daily), ritonavir (100 mg, twice daily), or bosentan (125 mg, twice daily) were well tolerated in healthy adult subjects and no significant safety concerns were observed in the study.

1.2.5.4. A Phase 1, Open-Label, Mass-Balance Study of Radiolabeled [^{14}C]-GS-4997 in Healthy Subjects (Study GS-US-223-1573)

Study GS-US-223-1573 was designed as an open-label, single center, single dose Phase 1 study to evaluate the metabolism, route of elimination, and PK characteristics of radiolabeled [^{14}C]-GS-4997 (18 mg) in 8 healthy male subjects.

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. GS-4997 metabolism involved oxidation, hydrolysis, *N*-dealkylation, methylation, and glucuronidation with *N*-dealkylation as a major pathway. No new safety concerns for GS-4997 18-mg in healthy subjects were identified.

1.2.5.5. A Phase 1, Open-Label, Parallel-Group, Single Dose Study to Evaluate the Pharmacokinetics of GS-4997 in Subjects with Normal and Impaired Hepatic Function (Study GS-US-223-1018)

Study GS-US-223-1018 was a Phase 1, open-label, parallel-group, single-dose study evaluating the safety, tolerability, and PK of GS-4997 in subjects with normal hepatic function and mild, moderate, or severe hepatic impairment. Up to 60 subjects were planned for enrollment in 1 of 3 hepatic impairment cohorts: Cohort 1 (moderate hepatic impairment), Cohort 2 (severe hepatic impairment), or Cohort 3 (mild hepatic impairment). Within each cohort, each subject with impaired hepatic function was matched for age (± 10 years), sex, and body mass index (BMI; ± 20%) with a control subject with normal hepatic function. Data from healthy controls could be used in > 1 cohort if a subject was an appropriate match for a subject with hepatic impairment in > 1 cohort. All subjects received a single oral dose of GS-4997 6 mg in a fed state on Day 1. The study conduct is completed and final data analysis is ongoing. Preliminary results are presented below.

Preliminary pharmacokinetic results from this study are available. There were no significant changes in GS-4997 exposure (C_{max}, AUC_{inf}) in subjects with mild or moderate hepatic impairment (CPT class A and B, respectively) as compared to healthy matched control subjects as indicated by geometric least squares mean ratios (GLSMRs) near 100% and 90% confidence intervals (CIs) between 70% and 143%. There was a slight increase in GS-4997 AUC_{inf} (42.65%) in subjects with severe hepatic impairment (CPT class C) as compared to the healthy

matched control subjects for that group (N=10). An ad hoc analysis was performed to compare GS-4997 exposures in subjects with severe hepatic impairment to pooled healthy volunteers from all cohorts (N=22) and the increase in AUC_{inf} was less (21.4% increase). The mean plasma protein binding of GS-4997 at 3 hours postdose was similar in subjects with normal hepatic function and mild and moderate hepatic impairment (5.3% unbound in each group) with slightly higher unbound fraction in subjects with severe hepatic impairment (7.0%). Plasma exposures (C_{max} and AUC) of the primary metabolite GS-607509 were decreased (up to 53%) in subjects with moderate and severe hepatic impairment. GS-607509 is an inactive metabolite, thus, decreases in GS-607509 exposure are not clinically relevant.

The slight increase in GS-4997 exposure (AUC) in subjects with severe hepatic impairment is less than what was observed with a strong CYP3A4 inhibitor (79% increase). The small increase in exposure observed with severe hepatic impairment is not considered clinically meaningful as the observed changes are less than or comparable to the expected effect of a moderate CYP3A4 inhibitor on GS-4997 exposure; moderate CYP3A4 inhibitors are currently allowed in all ongoing Phase 2 studies evaluating doses of GS-4997 of up to 18 mg once daily. GS-4997 has been evaluated at doses up to 100 mg in Phase 1 studies for up to 2 weeks and up to 18 mg in Phase 2 studies for up to 48 weeks and appears to be well tolerated. Based on the current safety/tolerability profile for GS-4997 over a wide dose/exposure range, the increase in GS-4997 exposure in subjects with severe hepatic impairment is unlikely to be clinically relevant at the doses currently being evaluated in Phase 2 studies (up to 18 mg once daily). Therefore, dose adjustments are not considered necessary in subjects with mild, moderate, or severe hepatic impairment.

1.2.5.6. A Phase 2, Double-blind, Placebo-controlled, Dose-ranging Study Evaluating the Efficacy, Safety, and Tolerability of GS-4997 in Subjects with Diabetic Kidney Disease (Study GS-US-223-1015)

Study GS-US-223-1015 is an ongoing Phase 2, double-blind, placebo-controlled, dose-ranging study evaluating the efficacy, safety, and tolerability of GS-4997 in subjects with DKD. Approximately 300 subjects with type 2 diabetes mellitus and Stage 3 or Stage 4 renal impairment and albuminuria receiving standard of care for DKD are planned to be randomized (1:1:1:1) to 1 of 4 treatment groups: GS 4997 2, 6, or 18 mg or matching placebo administered once daily for 48 weeks. The primary objective of this study is to determine the effect of GS-4997 on estimated glomerular filtration rate (eGFR) decline in subjects with DKD.

1.2.5.6.1. Preliminary Safety Analyses

Based on the database used for the DMC meeting on 11 November 2015 which included unblinded safety data from 332 subjects (data cut-off date of 6 October 2015), 207 subjects (62.3%) had at least 1 treatment-emergent AE during the study; 36 events in 29 subjects were considered by the investigator to be related to study drug; 79 events in 43 subjects were serious adverse events. The most frequently reported AEs were hyperkalemia (15 of 332 subjects [4.5%]), diarrhea, nausea (14 of 332 subjects each [4.2%]), back pain, hyperuricemia [13 subjects each [3.9%]), upper respiratory tract infection (12 subjects [3.6%]), acute kidney injury, hypertension (11 subjects each [3.3%]), constipation, edema peripheral, and

hypoglycemia (10 subjects each [3.0%]). Counting each subject once using the highest toxicity grade, Grade 3 and 4 AEs were reported for 31 and 6 subjects, respectively, and 1 death (grade 5) occurred (unobserved death, likely myocardial infarction in a patient with pre-existing coronary artery disease). Thirteen subjects had AEs leading to study drug discontinuation. Since the study is ongoing, treatment assignment for the AEs is not known.

1.2.5.7. A Phase 2, Dose-ranging, Randomized, Double-blind, Placebo-controlled Study of GS-4997 in Subjects with Pulmonary Arterial Hypertension (Study GS-US-357-1394)

Study GS-US-357-1394 is an ongoing Phase 2, randomized, double-blind, placebo-controlled, multicenter, dose-ranging study of GS-4997 in subjects with PAH receiving background stable PAH therapy. Approximately 120 subjects with a diagnosis of idiopathic PAH (IPAH), heritable PAH (HPAH), or PAH associated with connective tissue disease (PAH-CTD), congenital heart defects (repaired), drug and toxin use, or HIV infection are planned to be randomized 1:1:1:1 to receive either GS-4997 2, 6, or 18 mg or placebo, orally, once daily, for 24 weeks. Subjects also will continue receiving their background PAH therapy throughout the study. Subjects who complete the 24-week blinded treatment period will be eligible to continue (or initiate) treatment with GS-4997 at 2, 6, or 18 mg during the blinded, long-term treatment extension of this study. The primary objective of this study is to evaluate the effect of GS-4997 on pulmonary vascular resistance (PVR), as measured by right heart catheterization (RHC) in subjects with PAH.

As of 15 February 2016, 151 subjects had been randomized and 150 subjects have been treated with study drug or placebo for a median (min, max) of 16.1 (3.9, 48.1) weeks. A total of 101 subjects (67.3%) remain on treatment and 14 subjects (9.3%) have discontinued study drug. The most frequent reason for discontinuation of study drug was due to an AE (5 subjects, 3.3%), followed by investigator's discretion (4 subjects, 2.7%), and protocol violation, withdrawal of consent, and increase in dose of PAH medication or new PAH medication added (1 subject each, 0.7% each). Because the study is still ongoing and blinded, the treatment received by these subjects is unknown. The external DMC review of the unblinded safety data from 150 subjects resulted in no safety concerns.

1.2.5.8. A Phase 2, Randomized, Open Label Study Evaluating the Safety, Tolerability, and Efficacy of GS-4997 alone or in Combination with Simtuzumab (SIM) in Subjects with Nonalcoholic Steatohepatitis (NASH) and Fibrosis (F2-F3) (GS-US-384-1497)

Study GS-US-384-1497 is an ongoing, randomized, open-label, multicenter, Phase 2 study of GS-4997 evaluating the safety, tolerability, and biological activity of GS-4997 alone or in combination with simtuzumab (SIM) in subjects with NASH and liver fibrosis (stages F2-F3). Approximately 70 subjects are planned to be randomized 2:2:1:1:1 into 5 groups (Group A to Group E) to receive 1 of the following study drug regimens for 24 weeks: GS-4997 6 mg, orally, once daily (Group A), GS-4997 18 mg, orally, once daily (Group B), GS-4997 6 mg, orally, once daily + SIM 125 mg, SC, weekly (Group C), GS-4997 18 mg, orally, once daily + SIM 125 mg, SC, weekly (Group D) and SIM 125 mg, SC, weekly (Group E). The primary objective of this

study is to evaluate the safety and tolerability of GS-4997 alone or in combination with SIM in subjects with NASH and fibrosis stages F2-F3.

As of 31 March 2016, 72 subjects out of a planned 72 subjects with NASH and liver fibrosis have been randomized 2:2:1:1:1 to 1 of 5 treatment groups to receive GS-4997 alone or GS-4997 in combination with simtuzumab for 24 weeks. Open-label long term dosing will be considered with a regimen to be determined after all data are analyzed.

Preliminary Safety Analyses

Based on the database used for the DMC meeting on 26 January 2016 which included unblinded safety data from 20 subjects (data cut-off date of 22 Dec 2015), 4 subjects (20%) had at least 1 treatment-emergent AE during the study; none were considered by the investigator to be related to study drug; none were serious adverse events. The most frequently reported AEs were nausea (2 of 20 subjects [10%]), and amnesia (2 of 20 subjects [10%]). No AE of ≥ Grade 3 severity was reported and no adverse events led to the discontinuation of study drug.

1.3. Rationale for This Study

ASK1 is a serine/threonine kinase that is activated by oxidative stress and stimulates apoptotic, fibrogenic, and inflammatory pathways via p38 and JNK activation. In mouse models of non-alcoholic acute liver injury, ASK-1 has been shown to promote hepatocyte apoptosis and necrosis {Nakagawa et al 2011}. In an internal study, liver biopsies from 9 patients with severe AH were shown to have significantly higher p-p38 immunoreactivity (an indicator of ASK1 pathway activation) compared to biopsies from patients with NASH and PSC suggesting that ASK1 inhibition may be a potential therapeutic target in severe AH. GS-4997 is a small molecule, potent and selective inhibitor of ASK1. Inhibition of ASK1 with GS-4997 and resultant reduction of oxidative stress-induced hepatocyte apoptosis and necrosis, which are key mediators of AH pathogenesis {Ziol et al 2001} is hypothesized to improve efficacy and safety compared with corticosteroid treatment in patients with AH.

Mortality in AH is associated with acute loss of liver function and accompanying complications of advanced liver disease, including encephalopathy, sepsis, and/or MOF including renal failure.Data generated at Gilead have demonstrated that ASK1 inhibition does not directly reduce LPS-mediated signaling and cytokine release by macrophages, nor does it reduce oxidative burst by neutrophils. These pathways are considered important mechanisms in the pathophysiology of severe AH. However it is hypothesized that the hepatoprotective effects of GS-4997 via other mechanisms could translate into improved liver function and resultant improved mortality in severe AH. Early improvement in liver function is associated with better survival (eg, 1-6 months following presentation) {Louvet et al 2015, Louvet et al 2007}.Prednisolone is the standard of care for severe AH and has been shown to reduce systemic levels of cytokines (ie, TNF-α, IL-8) and reduce neutrophil activity (oxidative burst) in patients with severe AH {Taieb et al 2000}. The complementary mechanisms of action of GS-4997 and prednisolone suggest a potential benefit could be gained via their combination. Given the distinct mechanism of action of GS-4997 and the totality of the preclinical evidence of modulating

pathways involved in liver damage in AH, evaluating the combination of GS-4997 and prednisolone in patients with AH is of merit.

1.4. Rationale for Dose Selection

GS-4997 tablets will be administered orally once daily at a dose of 18 mg.

In preclinical models of DKD, PAH, NASH, and acute acetaminophen overdose, maximal efficacy is achieved with a dose that provides near complete suppression of the ASK1 pathway in target tissues (> EC_{95}). For this Phase 2 proof-of-concept study, the dose selected (18 mg once daily) is projected to provide a plasma concentration that is expected to cause near complete inhibition of ASK1 (> EC_{95}) at trough based on an ex vivo CXCL1 assay in human whole blood (CXCL1 EC_{95} = 130 ng/mL) to maximize potential for efficacy in this severe acute indication. This dose selection is supported by a preliminary review of unblinded biomarker data from an ongoing double-blind Phase 2 study in DKD (GS-US-223-1015) evaluating doses of 2 mg, 6 mg, and 18 mg of GS-4997 once daily.

GS-4997 has been evaluated at doses up to 100 mg in Phase 1 studies and up to 18 mg in Phase 2 studies and, based on currently available blinded and unblinded safety data, is well tolerated. Based on in vitro and in vivo DDI results with probe drugs, GS-4997 PK is not expected to be significantly altered when coadministered with prednisolone as planned for this Phase 2 study. In the Phase 1 hepatic impairment study (GS-US-223-1018), there were minimal to modest changes in GS-4997 PK across varying degrees of hepatic impairment (mild, moderate, and severe). A 43% increase in GS-4997 exposure (AUC) in subjects with severe hepatic impairment is less than what was observed in Study GS-US-223-1434 with the strong CYP3A4 inhibitor ritonavir (79% increase). The small increase in exposure observed with severe hepatic impairment is not considered clinically meaningful as the observed changes are less than or comparable to the expected effect of a moderate CYP3A4 inhibitor on GS-4997 exposure; moderate CYP3A4 inhibitors are currently allowed in all ongoing Phase 2 studies evaluating doses of GS-4997 of up to 18 mg once daily. Based on the current safety/tolerability profile for GS-4997 at doses more than 5-fold higher than the dose proposed for this study, the increase in GS-4997 exposure in subjects with severe hepatic impairment is not considered clinically relevant and does not require dose adjustment. Taken together, these data support evaluation of GS-4997 18 mg once daily in subjects with severe AH.

1.5. Risk/Benefit Assessment for the Study

The primary risk of study participation relates to the proposed initiation of prednisolone ± GS-4997 prior to the histologic confirmation of alcoholic hepatitis. The rationale for this approach is two-fold. First, in many centers, performance of liver biopsy in subjects with suspected severe alcoholic hepatitis is not standard of care prior to initiating treatment, typically with prednisolone. Second, the planned approach will allow the rapid initiation of therapy which is deemed vital to optimize outcomes in subjects with severe alcoholic hepatitis. Importantly, a clinical diagnosis of alcoholic hepatitis (as per standard of care) and contraindications to prednisolone (e.g. infection) will be excluded in all subjects prior to starting treatment.

Another related risk of study participation is that some subjects who do not have alcoholic hepatitis will be exposed to prednisolone ± GS-4997 therapy for up to 7 days before the diagnosis is refuted on biopsy. Based on prior studies, an estimated 20% of subjects who are suspected of severe alcoholic hepatitis do not have confirmation of the diagnosis on biopsy. As mentioned above, the initiation of prednisolone in subjects with suspected alcoholic hepatitis without performance of a liver biopsy is standard practice in many centers. The plan to discontinue treatment in subjects in whom the biopsy does not confirm alcoholic hepatitis is a potential benefit of study participation because it will limit exposure to prednisolone, which has toxicities including infection and worsened glycemic control. The risk of short-term exposure to GS-4997 is low based on the results of prior Phase 1 and 2 studies (as outlined in Section 1.2.5), including the hepatic impairment study (GS-US-223-1018) that included subjects with mild, moderate, and severe hepatic dysfunction.

In view of the potential for GS-4997 to reduce major mechanisms of liver injury in subjects with severe alcoholic hepatitis, the limitations in available therapy, existing safety data, and the proposed study design to carefully monitor patient safety, the benefit/risk balance for this study is considered positive.

1.6. Compliance

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

2. OBJECTIVES

The primary objective of this study is:

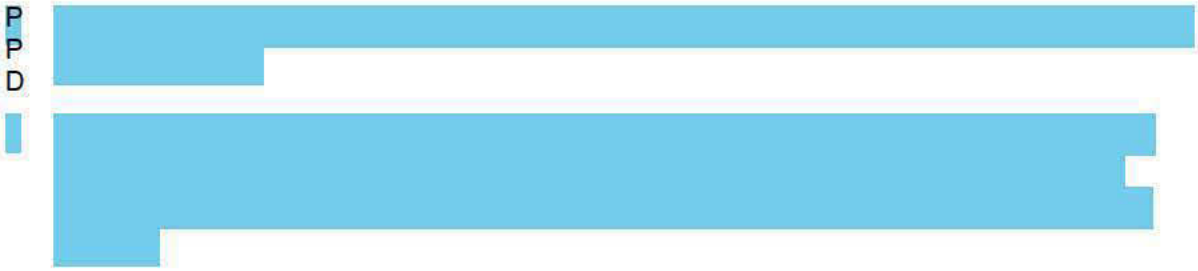
- To evaluate the safety and tolerability of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe AH

The secondary objectives of this study are as follows:

- To assess changes in hepatic synthetic function [liver biochemistry, Model for End-Stage Liver Disease [MELD] score, Child-Pugh score, the Lille model, Maddrey's Discriminant Function (DF), and PPD
- To assess mortality at 28 days, Weeks 12 and Week 24
- To determine the incidence of liver transplantation;
- To determine the incidence of hepatorenal syndrome (HRS);
- To determine the incidence of infection;
- To assess length of hospital stay;

The exploratory objectives of this study are:

- PPD

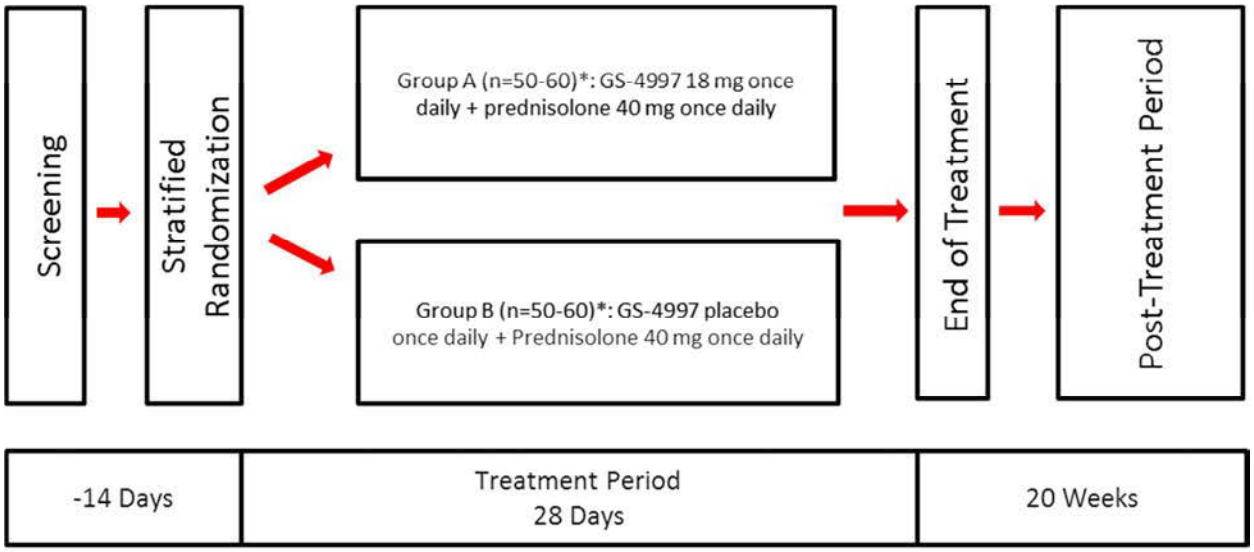


3. STUDY DESIGN

3.1. Study Design

The overall study design is presented graphically in Figure 3-1.

Figure 3-1. Overall Study Design



* Up to 60 subjects will be randomized leading to 50 subjects in each arm for the full analysis set (ie, histologically-proven severe AH and took at least one dose of study drug)

3.2. Treatment Plan and Regimen

This is a Phase 2 double blind, randomized study evaluating the safety, tolerability, and biological activity of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe, histologically-confirmed AH.

Up to 120 subjects will be randomized in a 1:1 ratio to 1 of 2 treatment groups:

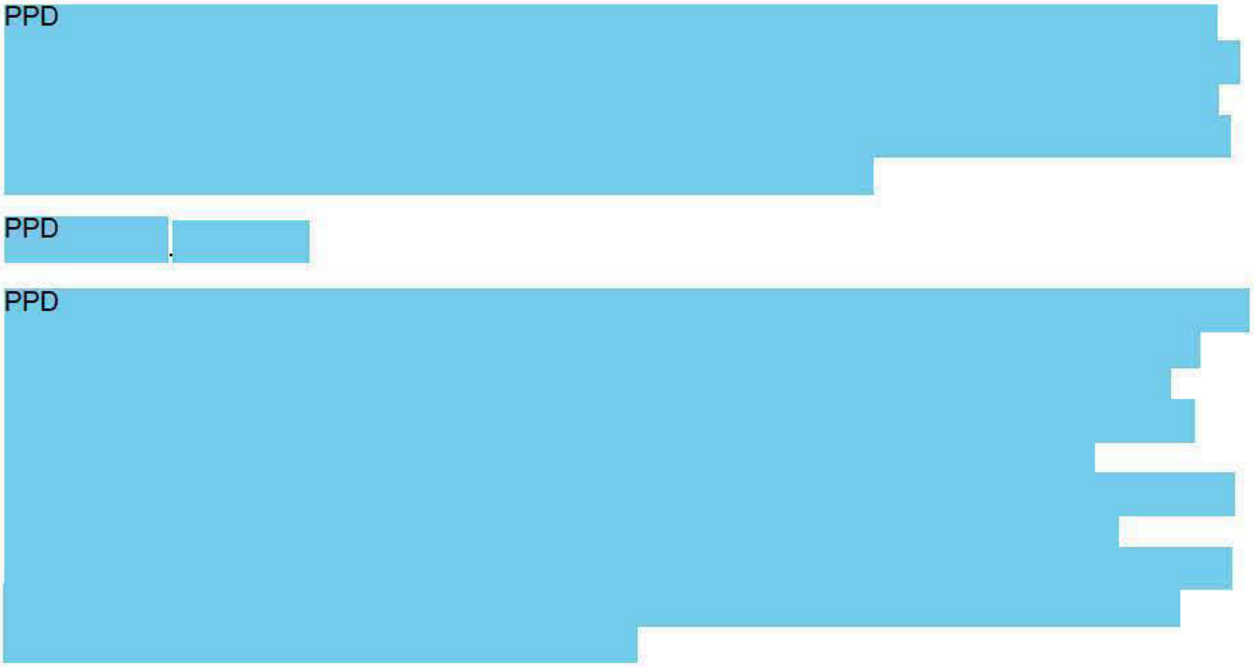
- Group A (n = 50-60): GS-4997 18 mg once daily + prednisolone 40 mg once daily for 28 days
- Group B (n = 50-60): GS-4997 placebo once daily + prednisolone 40 mg once daily once daily for 28 days

Randomization will be stratified by MELD < 25 or ≥25 at screening.

The full analysis set will comprise 100 subjects but up to 120 subjects will be randomized to account for subjects in whom AH is not confirmed on liver biopsy. Full analysis set includes those randomized and dosed with histologically-proven severe AH. Safety data will be reported for all subjects who take study drug.

All subjects with severe AH should complete screening, on-treatment, and post-treatment assessments as outlined in Appendix 2. If the subject meets criteria for discontinuation of treatment (see Section 6.8 of this protocol), the subject should complete an End of Treatment (EoT) visit as soon as possible after all study drugs are discontinued and attend post-treatment visits as outlined in Appendix 2. If biopsy results are not consistent with AH, the subject must stop study drugs within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible and have a Follow Up visit 30 days after stopping drug, and be withdrawn from the study.

Intensive PK Sub-study



Stopping Rules for Individual Subjects

Administration of all study drugs may be discontinued due to a clinical or laboratory adverse event. There is no option for GS-4997/placebo dose reduction, but GS-4997/placebo may be interrupted due to drug-related toxicity following discussion with the Medical Monitor. If GS-4997/placebo is interrupted, it may be restarted on a case by case basis after discussion with the Medical Monitor. GS-4997/placebo should be stopped if AST/ALT increase to >5x baseline/Day 1/nadir (if confirmed on repeat testing) or if bilirubin increases to >3x baseline/Day 1/nadir (if confirmed on repeat testing). Prednisolone can be dose reduced/interrupted at the discretion of the investigator but this should also be discussed with the Medical Monitor. Subjects are allowed to take one study drug and not the other.

3.3. Biomarker Testing

3.3.1. Biomarker Samples to Address the Study Objectives

PPD

. Stool samples may be used for microbiome analysis. The specific analyses will include, but will not be limited to, the biomarkers and assays listed below in Table 3-1. Because biomarker science is a rapidly evolving area of investigation, and adverse events in particular are difficult to predict, it is not possible to specify prospectively all tests that will be done on the specimens provided. The testing outlined is based upon the current state of scientific knowledge. It may be modified during or after the end of the study to remove tests no longer indicated and/or to add new tests based upon the growing state of the art knowledge. If a test will no longer be performed because the assay is not robust, sample collection may also be withdrawn to reduce burden on subjects.

Specimens will be collected from all subjects. Samples will be destroyed no later than 15 years after the end of the study. The specimen storage period will be in accordance with the IRB/IEC/EC-approved Informed Consent Form (ICF) and applicable laws (eg, health requirements).

Table 3-1. Biomarkers

Biomarker Focus	Planned Biomarkers	Rational	Specimen Collected
Liver tissue surrogate marker panel	ELF Test	Assess potential changes in liver fibrosis due to treatment	Blood
Liver tissue damage marker	Serum LOXL2	Assess potential changes in liver tissue damage due to treatment	Blood
Apoptosis and Necrosis markers	Circulating Cytokeratin 18 (CK18) fragments, M30 for apoptosis and M65 for all dying cells)	Determine changes in liver cell death due to treatment	Blood
Inflammatory involvement	Serum cytokines	Determine changes in the inflammatory status due to treatment	Blood
Infection	Lipopolysaccharide (LPS)/endotoxin	Assess changes in gut barrier function due to treatment	Blood
Infection	Procalcitonin	Assess changes in gut barrier function due to treatment	Blood

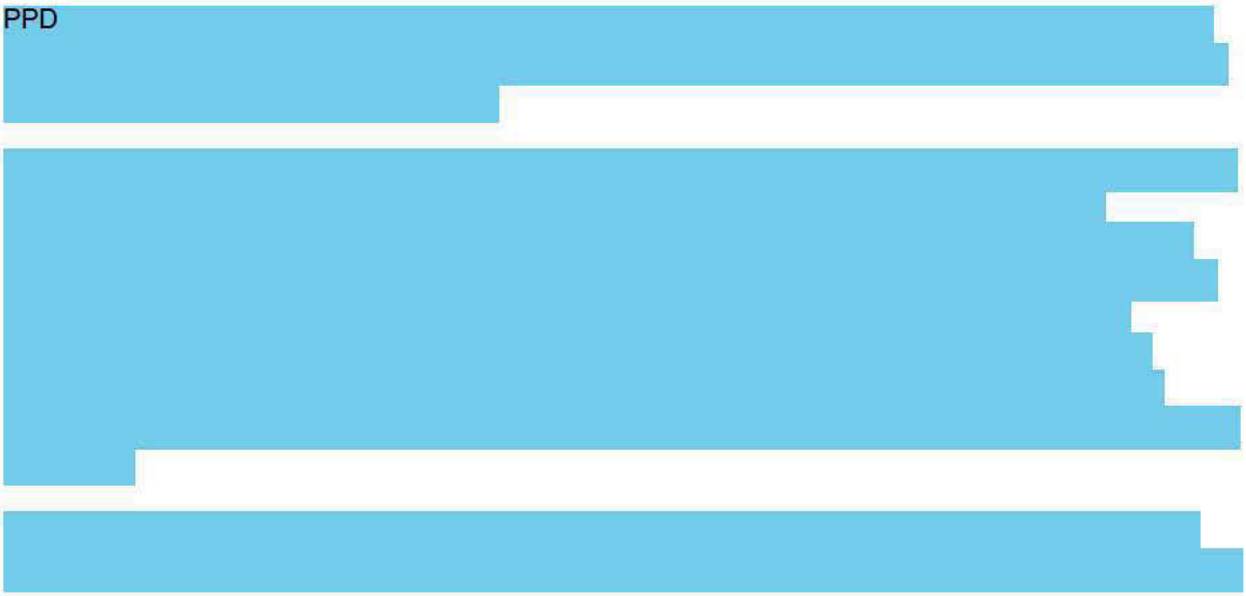
Biomarker Focus	Planned Biomarkers	Rational	Specimen Collected
Metabolome	Circulating metabolite profiles (open platform)	Assess changes in the metabolome due to treatment	Blood
ASK1 pathway activity	phospho-p38*	blood marker of ASK1 pathway activity to monitor PD effects	Blood
Intestinal microbial composition	Stool microbiome determined by 16S ribosomal DNA sequencing	Assess changes in microbial populations due to treatment	Stool
ASK1 pathway activity	Focused panel gene expression (Nanostring)	Assess gene expression in disease at baseline	Liver
ASK1 pathway activity	phospho-p38 IHC	Assess ASK1 pathway activation at baseline	Liver
FXR Pathway Activity	FGF19	Assess FXR pathway activity to inform potential combinations	Blood
Circulating Micro RNA	Serum miRNA	Assess changes in circulating miRNA associated with liver disease due to treatment	Blood

* Biomarker blood sample phospho-p38 will only occur at select US study sites.

For sampling procedures, storage conditions, and shipment instructions, see the Central Laboratory Services Manual.

3.3.2. Biomarker Samples for Optional Future Research

PPD



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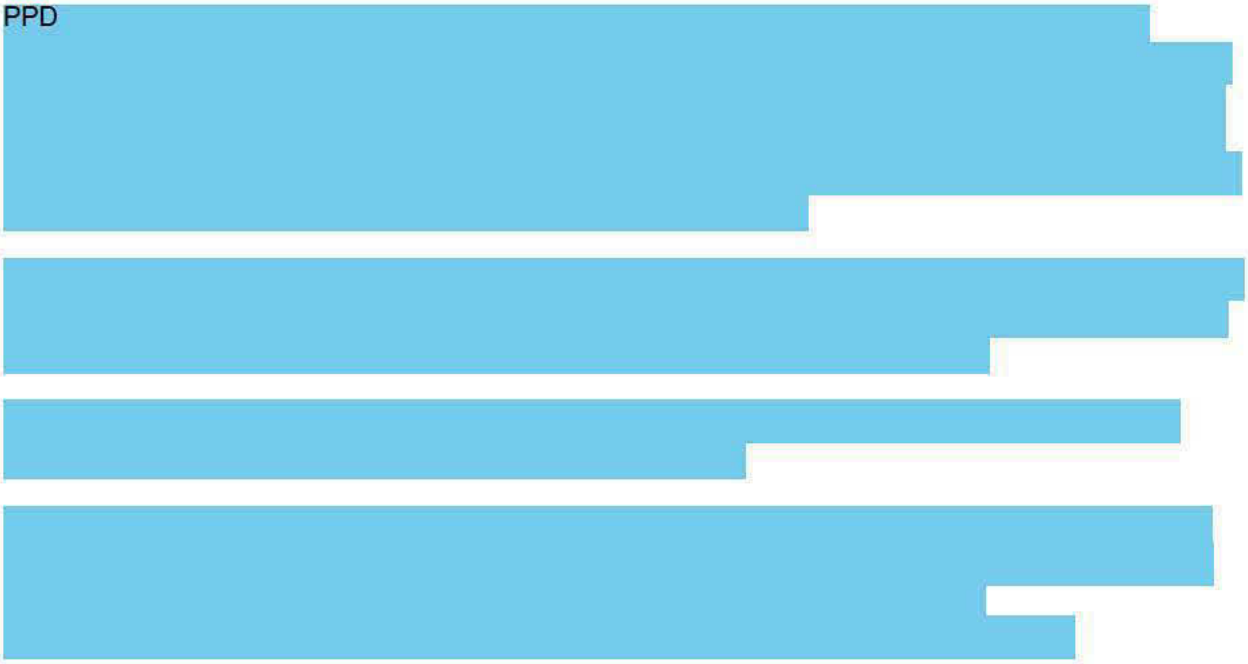

3.3.3. Biomarker Samples for Optional Genomic Research

PPD

PPD

4. SUBJECT POPULATION

4.1. Number of Subjects and Subject Selection

The full analysis set will comprise 100 subjects, males and non-pregnant, non-lactating females between 18-70 years of age with histologically-confirmed severe alcoholic hepatitis (Maddrey's DF ≥ 32). Up to 20 additional subjects will be randomized to account for subjects in whom alcoholic hepatitis is not histologically-confirmed.

4.2. Inclusion Criteria

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

1) Males and non-pregnant, non-lactating females between 18-70 years of age, inclusive based on the date of the screening visit;

2) Willing and able to give informed consent prior to any study specific procedures being performed. In subjects with hepatic encephalopathy (HE), which may impair decision-making, consent will be obtained per hospital procedures (eg, by legal Authorized Representative);

3) Clinical diagnosis of severe AH based on:

   a) History of excessive alcohol consumption during the past 3 months (average of >40 g/d of alcohol for women and >50 g/d for men);

   b) Onset of jaundice within the past 3 months;

   c) Maddrey's DF ≥ 32 at screening;

4) All female subjects of childbearing potential must agree to use a highly effective method of contraception during intercourse from the screening visit throughout the study period and for 90 days following the last dose of study drug. If females utilize hormonal agents as one of their contraceptive methods, the same hormonal methods must have been used for at least 1 month before study dosing. Females on hormonal methods must also utilize a barrier method as another form of contraception as described in Appendix 3;

5) Male subjects must refrain from sperm donation from screening through at least 90 days following the last dose of study drug;

6) Male subjects must agree to use condoms during intercourse from screening through study completion and for 90 days following the last dose of study drug;

7) Female subjects must refrain from egg donation or harvest for 90 days after last dose of study drug;

8) Willing and able to comply with scheduled visits, drug administration plan, laboratory tests, other study procedures, and study restrictions

4.3. Exclusion Criteria

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

1) Pregnant or lactating females;

2) Other causes of liver disease including chronic hepatitis B (hepatitis B surface antigen [HBsAg] positive), chronic hepatitis C (HCV RNA positive), acetaminophen hepatotoxicity, biliary obstruction, and autoimmune liver disease (subjects with fatty liver due to coexistent metabolic syndrome will not be excluded);

3) Serum aspartate aminotransferase (AST) >400 U/L or alanine aminotransferase (ALT) >300 U/L;

4) MELD >30 at screening;

5) Grade 4 Hepatic Encephalopathy (HE) by West Haven criteria;

6) Concomitant or previous history of hepatocellular carcinoma;

7) History of liver transplantation;

8) HIV Ab positive;

9) Uncontrolled sepsis;

10) Uncontrolled gastrointestinal (GI) bleeding or controlled GI bleeding within 7 days of screening that was associated with shock or required transfusion of more than 3 units of blood;

11) Type 1 hepatorenal syndrome (HRS) or renal failure defined as a serum creatinine >250 μmol/L (> 2.8 mg/dL) or the requirement for renal replacement therapy;

12) Subjects dependent on inotropic (eg, epinephrine or norepinephrine) or ventilatory support (ie, endotracheal intubation or positive-pressure ventilation);

13) Portal vein thrombosis;

14) Acute pancreatitis;

15) Cessation of alcohol consumption for more than 2 months before baseline/Day 1;

16) Severe associated disease (eg, cardiac failure, acute myocardial infarction, severe cardiac arrhythmias, severe pulmonary disease, neurologic disease,) that may lead to premature mortality within the study period;

17) Malignancy within the 2 years prior to screening, with the exception of specific cancers that have been cured by surgical resection (basal cell skin cancer, etc). Subjects under evaluation for possible malignancy are not eligible.

18) Positive urine screen for amphetamines, cocaine or opiates (ie, heroin, morphine) at screening. Subjects on stable methadone or buprenorphine maintenance treatment for at least 6 months prior to screening may be included in the study. Subjects with a positive urine drug screen due to prescription opioid-based medication are eligible if the prescription and diagnosis are reviewed and approved by the investigator;

19) Treatment with immunosuppressive drugs (eg, systemic corticosteroids, budesonide, tacrolimus, sirolimus, cyclosporine, azathioprine, mycophenolate mofetil, and methotrexate), pentoxifylline, or N-acetylcysteine (NAC) within 6 month of screening;

20) Use of the following CYP3A4 inhibitors (clarithromycin, conivaptan, grapefruit juice, itraconazole, ketoconazole, nefazodone, posaconazole, bupenorphine/naloxone, telithromycin, voriconazole) or CYP3A4 inducers (carbamazepine, phenytoin, rifampin, St. John's Wort) within 2 weeks of baseline;

21) Active ocular herpes simplex;

22) Any laboratory abnormality or condition that, in the investigator's opinion, could adversely affect the safety of the subject or impair the assessment of study results;

23) Participation in another investigational study of a drug or device within 1 month prior or within 5 half-lives of the prior investigational agent (whichever is longer) prior to screening;

24) Concurrent participation in another therapeutic clinical study;

25) Known hypersensitivity to the study drugs (GS-4997 and prednisolone), the metabolites, or formulation excipient;

26) Presence of any condition that could, in the opinion of the investigator, compromise the subject's ability to participate in the study, such as history of substance abuse other than alcohol use or a psychiatric or medical condition;

27) Unavailable for follow-up assessment or concern for subject's compliance with the protocol procedures

5. INVESTIGATIONAL MEDICINAL PRODUCTS

5.1. Randomization, Blinding and Treatment Codes

This is a randomized, double blind study. An Interactive Web Response System (IWRS) will be used for centralized randomization and treatment assignment. Randomization will be stratified by MELD < or ≥ 25 at screening. Within each of the strata, subjects will be randomized in a 1:1 ratio to receive GS-4997 + prednisolone or GS-4997 placebo + prednisolone .

Investigative site personnel will obtain the subject's identification number and study drug assignment from the IWRS.

Study drug will be dispensed by the study pharmacist, or designee.

5.1.1. Procedures for Breaking Treatment Codes

In the event of a medical emergency where breaking the blind is required to provide medical care to the subject, the investigator may obtain treatment assignment directly from the IWRS system for that subject. Gilead recommends but does not require that the investigator contacts the Gilead medical monitor before breaking the blind. Treatment assignment should remain blinded unless that knowledge is necessary to determine subject emergency medical care. The rationale for unblinding must be clearly explained in source documentation and on the electronic case report form (eCRF), along with the date on which the treatment assignment was obtained. The investigator is requested to contact the Gilead medical monitor promptly in case of any treatment unblinding.

Blinding of study treatment is critical to the integrity of this clinical trial and therefore, if a subject's treatment assignment is disclosed to the investigator, the subject will have study treatment discontinued. All subjects will be followed until study completion unless consent to do so is specifically withdrawn by the subject.

Gilead Drug Safety and Public Health (DSPH) may independently unblind cases for expedited reporting of suspected unexpected serious adverse reactions (SUSARs).

5.2. Description and Handling of Study Drugs

5.2.1. Formulation

5.2.1.1. GS-4997 18 mg and GS-4997 Placebo to Match (PTM)

GS-4997 will be supplied as 18 mg strength round, plain-faced, white film-coated tablets. In addition to the active ingredient, GS-4997 tablets contain the following commonly used inactive ingredients: CCI [redacted]

The matching placebo tablets are identical in physical appearance and contain the same inactive ingredients as the GS-4997 18 mg tablets.

5.2.1.2. Prednisolone

Commercially available prednisolone will be used for the study. Further information regarding formulation is available in the Prescribing Information for commercial product.

5.2.2. Packaging and Labeling

5.2.2.1. GS-4997 18 mg and GS-4997 PTM

GS-4997 18 mg and GS-4997 PTM tablets are packaged in white high density polyethylene (HDPE) bottles. Each bottle contains 30 tablets, silica gel desiccant and polyester packing material. Each bottle is enclosed with a white, continuous-thread, child-resistant polypropylene screw cap with an induction-sealed and aluminum-faced liner.

Study drug to be distributed to centers in the US and other participating countries shall be labeled to meet applicable requirements of the United States Food and Drug Administration (FDA), EU Guideline to Good Manufacturing Practice - Annex 13 (Investigational Medicinal Products), and/or other local regulations.

5.2.2.2. Prednisolone

Commercially available prednisolone 10 mg tablets will be used for the study.

5.2.3. Storage and Handling

5.2.3.1. GS-4997 and GS-4997 PTM

GS-4997 18 mg and GS-4997 PTM tablets should be stored at controlled room temperature of 25°C (77 °F); excursions are permitted between 15 °C and 30 °C (59 °F and 86 °F). Storage conditions are specified on the label. Until dispensed to the subjects, all bottles of study drug should be stored in a securely locked area, accessible only to authorized site personnel. To ensure the stability and proper identification, study drug should not be stored in containers other than the containers in which they were supplied.

Consideration should be given to handling, preparation, and disposal through measures that minimize drug contact with the body. Appropriate precautions should be followed to avoid direct eye contact or exposure when handling.

5.2.3.2. Prednisolone

Commercially available prednisolone 10 mg tablets will be used for the study. Further information regarding storage and handling are available in the Prescribing Information for commercial product.

5.3. Dosage and Administration of Study Drugs

5.3.1. GS-4997

GS-4997 18 mg and GS-4997 PTM tablets will be provided by Gilead Sciences. Once daily, at approximately the same time each morning, the study drug should be taken orally with water, swallowed whole, and with or without food. A dose will be considered missed if the subject cannot take the dose within 8 hours of their regular dosing time. If a subject misses a dose, the subject should take their next dose at the regular dosing time. Please note that study drug should not be taken on mornings of sparse PK and biomarker assessments as outlined in Appendix 2. The dose should be taken as soon as possible after the trough PK and trough biomarker assessments are completed.

5.3.2. Prednisolone

Commercially available prednisolone 10 mg tablets will be provided by Gilead Sciences. Subjects will take 40 mg prednisolone (4 x 10 mg) orally with water, once a day, with or without meals. The study drug should be swallowed whole and taken at approximately the same time each day. A dose will be considered missed if the subject cannot take the dose within 8 hours of their regular dosing time. If a subject misses a dose, the subject should take their next dose at the regular dosing time.

5.4. Prior and Concomitant Medications

In vitro data suggests GS-4997 has the potential to inhibit P-glycoprotein (P-gp). GS-4997 has the potential to increase the exposure of sensitive P-gp substrates (eg, aliskiren, dabigatran etexilate, digoxin, fexofenadine, ranolazine). In a drug-drug interaction study of GS-4997 co-administered with digoxin (0.5 mg), exposure of digoxin (AUC) was increased by less than 30%. GS-4997 may increase the exposure of other P-gp substrates; however, the clinical relevance of such interactions has not been determined. Co-administration of P-gp substrates with study drug is allowed, with specific guidance provided for the following:

- Digoxin: Follow digoxin level at baseline and at the Week 1 visit with digoxin level checks during the study period per investigator discretion (as frequently as needed).
- Dabigatran etexilate: Consult Medical Monitor
- Ranolazine: Monitor for side effects
- Aliskiren: Use with caution, dose may need to be reduced

All concomitant medication will be recorded in the source documents. This includes concomitant medications taken within 30 days prior to screening and any taken during the study to the end of the follow-up period. Enteral and parenteral nutritional products administered during the study will be recorded on eCRFs.

5.5. Prohibited Medications

The use of any investigational medication or device in the 30 days prior to screening and through the study is prohibited. Administration of pentoxifylline or NAC during the study period is also prohibited.

Co-administration of strong CYP3A4 inhibitors may increase GS-4997 exposure. In a drug-drug interaction study of GS-4997 co-administered with ritonavir (strong CYP3A4 inhibitor), GS-4997 exposures were significantly increased. Therefore, co-administration of the following CYP3A4 inhibitors with study drug is prohibited: clarithromycin, conivaptan, grapefruit juice, itraconazole, ketoconazole, nefazodone, posaconazole, telithromycin, and voriconazole.

Co-administration of strong CYP3A4 inducers may decrease GS-4997 exposures. In a drug-drug interaction study of GS-4997 co-administered with repeat dose rifampin (strong CYP3A4 inducer), GS-4997 exposures were significantly reduced. Therefore, co-administration of the following CYP3A4 inducers with study drug is prohibited: carbamazepine, phenytoin, rifampin, St. John's wort.

5.6. Study Drug Accountability

The investigator or designee (eg, pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgement of receipt of each shipment of study drug (quantity and condition), subject administration and dispensing records, and returned or destroyed study product. Administration and dispensing records will document quantities received from Gilead Sciences and quantities administered/dispensed to subjects, including the lot number, date administered/dispensed, subject identification number, subject initials, and the initials of the person dispensing the medication. All used and unused study drug administered/dispensed to subjects must be returned to the site.

Investigational Drug Accountability records will be provided to each study site to:

- Record the date and quantity of study drug received
- Record the date, subject number, subject initials, and the study drug assigned
- Record the date, quantity of used and unused study drug. Dispensing records will include the initials of the person recording the information.

5.6.1. Investigational Medicinal Product Return or Disposal

At the start of the study, the study monitor will evaluate each study center's study drug disposal procedures and provide appropriate instruction for return or destruction of unused study drug supplies. If the site has an appropriate Standard Operating Procedure (SOP) for drug destruction, the site may destroy used (empty bottles) and unused study drug supplies performed in accordance with the site's (hospital/pharmacy) SOP. If the site does not have acceptable procedures in place for drug destruction, arrangements will be made between the site and

Gilead Sciences (or Gilead Sciences' representative) for return of unused study drug supplies. A copy of the site's SOP will be obtained for central files. Where possible, study drug will be destroyed at the site. Upon study completion, a copy of the Investigational Drug Accountability records must be filed at the site. Another copy will be returned to Gilead Sciences. If drug is destroyed on site, the investigator must maintain accurate records for all study drug bottles destroyed. Records must show the identification and quantity of each unit destroyed, the method of destruction, and person who disposed of the drug. All study drug records must be maintained at the site and copies must be submitted to Gilead Sciences at the end of the study.

6. STUDY PROCEDURES

The study procedures to be conducted for each subject enrolled in the study are presented in tabular form in Appendix 2 and described in the text that follows.

The site must document any deviation from protocol procedures and notify the sponsor or contract research organization (CRO).

6.1. Subject Enrollment and Treatment Assignment

It is the responsibility of the investigator to ensure that subjects are eligible to participate in the study prior to enrollment and throughout the study.

Documentation of the personally signed and dated informed consent of each subject, using the study-specific ICF, is required before initiating the screening process. In subjects with Hepatic Encephalopathy (HE), which may impair decision-making, consent will be obtained per hospital procedures (eg, by Legally Authorized Representative).

After written informed consent has been obtained and eligibility to participate established, investigative site personnel will obtain the subject's identification number and study drug assignment from the IWRS.

6.2. Pretreatment Assessments

6.2.1. Screening Visit

Subjects will be screened within 14 days prior to randomization to determine eligibility for participation in the study.

The interval between hospitalization and Baseline/Day 1 must be ≤ 14 days. This window may be extended under specials circumstances with explicit approval from the Medical Monitor.

Subjects who fail to meet eligibility criteria due to an abnormal laboratory result may undergo re-testing of the abnormal analyte once during the screening window. This will be done at the discretion of the investigator with prior approval of the Medical Monitor.

Subjects should be instructed to fast (no food or drink, except water), starting from midnight (00:00) or earlier, as appropriate, on the evening prior to the screening visit to ensure an approximate 8-hour fast prior to the blood sample collection under fasting condition the next morning.

Day -14 to Day 0

The following will be performed and documented at screening:

- Obtain written informed consent
- PPD
- PPD
- Obtain medical history
- Review and record whether the subject has met inclusion/exclusion criteria
- AUDIT / SADQ questionnaire
- Assess alcohol consumption
- Complete physical examination including, vital signs, body weight, and height
- Record all concomitant medications that the subject has taken within 30 days prior to screening
- Perform standard 12-lead ECG
- Collect urine samples for urine drug screen and culture
- Assessments for hepatic encephalopathy, ascites, Hepatorenal Syndrome (HRS), and infection
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
  - Serum Pregnancy Test (only for females subjects of childbearing potential)
  - Blood for Biomarkers

- HBV, HCV, and HIV serology
- Blood cultures
- Blood glucose and insulin (fasting)
- Lipid Profile (fasting)

- Perform chest x-ray
- Perform liver ultrasound (it is acceptable to use a liver ultrasound previously performed during the current hospitalization)
- Perform paracentesis (ascitic fluid tap) with ascitic fluid cell count, differential, and culture if clinically-detectable ascites is present
- Perform liver biopsy

  Note: Can be performed up to 7 days post Baseline/Day 1. Liver biopsy obtained during the current hospital admission prior to screening is acceptable. However, if biopsy results are not consistent with AH, the subject will stop study drug within 3 days of receipt of liver biopsy result, attend an End of Treatment (EoT) visit soon as possible, have a 30-day safety follow-up visit , and be withdrawn from the study.
- Record any serious adverse events and all adverse events occurring after signing of the consent form

From the time of obtaining informed consent through the first administration of investigational medicinal product, record all serious adverse events (SAEs), as well as any adverse events related to protocol-mandated procedures on the adverse events case report form (eCRF). All other untoward medical occurrences observed during the screening period, including exacerbation or changes in medical history are to be captured on the medical history eCRF. See Section 7 Adverse Events and Toxicity Management for additional details.

Subjects must meet all of the inclusion criteria and none of the exclusion criteria prior to randomization into the study.

6.2.2. Baseline / Day 1 Assessments and Randomization

Subjects eligible for randomization at Day 1 should be instructed to fast (no food or drink, except water), starting from midnight (00:00) or earlier, as appropriate, on the evening prior to the baseline/Day 1 visit to ensure an approximate 8-hour fast prior to the blood sample collection under fasting condition the next morning.

After review of inclusion and exclusion criteria to confirm continued eligibility, subjects will be randomized to study drug assignment and receive their Subject Identification Number via the IWRS prior to their first dose of study drug.

Randomization will be stratified by MELD < or ≥ 25 at screening.

The following will be performed and documented at the randomization visit prior to dosing:

- Review inclusion/exclusion criteria and confirm continued eligibility
- QoL Questionnaire (CLDQ)
- Assess alcohol consumption
- Complete physical examination including, vital signs, and body weight
- Perform standard 12-lead ECG
- Assessments for hepatic encephalopathy, ascites, HRS and infection
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
  - Blood for Biomarkers
  - ELF™ Test and LOXL2
  - Blood glucose and insulin (fasting)
  - Hemoglobin A1c
  - Lipid Profile (fasting)
  - Sparse PK Sample

- PPD
- PPD

- Urine pregnancy test (for females of childbearing potential)
- Collect stool for microbiome testing
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring after signing of the consent form
- Randomization via IWRS
- Administer or dispense GS-4997 and prednisolone, and provide subject with dosing instruction on appropriate dosing and administration

6.3. Treatment Assessments

6.3.1. Week 1 Visit (±3 Days)

During the Week 1 safety visit the following will be performed and documented:

- Review of liver biopsy results if unavailable during the screening period and reassess study entry criteria.
- Assess alcohol consumption
- Complete physical examination including, vital signs
- Assessments for hepatic encephalopathy, ascites, HRS, and infection
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR

— Blood for biomarkers

— Sparse PK Sample

— PPD [redacted]

- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit
- Administer or dispense prednisolone, and provide subject with dosing instruction on appropriate dosing and administration.
- PPD [redacted]
- Assess for Study Drug adherence
- Perform study drug accountability

6.3.2. Week 2 Visit (±3 Days)

During the Week 2 safety visit, the following will be performed and documented:

- Assess alcohol consumption
- Complete physical examination including vital signs
- Assessments for hepatic encephalopathy, ascites, HRS and infection
- Obtain blood samples for:

  — Hematology

  — Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)

  — Coagulation Tests: PT, PTT, and INR

  — Blood for biomarkers

  - PPD
  - Sparse PK Sample
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit
- PPD
- Assess for Study Drug adherence
- Perform study drug accountability

6.3.3. Week 3 Visit (±3 Days)

During the Week 3 safety visit, the following will be performed and documented:

- Assess alcohol consumption
- Complete physical examination including vital signs
- Assessments for hepatic encephalopathy, ascites, HRS and infection
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit
- Administer or dispense prednisolone, and provide subject with dosing instruction on appropriate dosing and administration

- PPD [redacted]
- Assess for Study Drug adherence
- Perform study drug accountability

6.3.4. Week 4 / End of Treatment Visit (EoT) (±3 days)

If the subject meets criteria for discontinuation of treatment (see Section 6.8 of this protocol), the subject should complete an End of Treatment visit as soon as possible after all study drugs are discontinued and attend post-treatment visits as outlined in Appendix 2. Subjects that have their scheduled Week 4 visit prior to Day 28 will require a phone contact between Study Days 28-35 to assess mortality and liver transplantation.

Subjects should be instructed to fast (no food or drink, except water), starting from midnight (00:00) or earlier, as appropriate, on the evening prior to the Week 4 visit to ensure an approximate 8-hour fast prior to the fasted blood sample collection the next morning.

Subjects should be instructed **not to take** study drug in the morning of the Week 4 Visit. The dose should be taken as soon as possible after the trough PK (Sparse PK sample collected prior to dosing) and biomarker assessments are completed.

The following will be performed and documented at this visit:

- QoL Questionnaire (CLDQ)
- Assess alcohol consumption
- Complete physical examination including, vital signs, and body weight
- Perform standard 12-lead ECG
- Assessments for hepatic encephalopathy, ascites, HRS, and infection.
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR

  - Blood for Biomarkers
  - ELF™ Test and LOXL2
  - Blood glucose and insulin (fasting)
  - Lipid Profile (fasting)
  - Sparse PK Sample
  - PPD [redacted]

- Urine pregnancy test (for females of childbearing potential)
- Collect stool for microbiome testing
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit
- Assess for Study Drug adherence
- Perform study drug accountability

6.3.5. Follow Up Visit (±5 Days)

Subjects whose liver biopsy results are not consistent with AH, must stop study drugs within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible and have a Follow Up visit 30 days after their last dose of study drug, and be withdrawn from the study.

- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)

6.4. Pharmacokinetic Assessments

6.4.1. Sparse PK

Blood samples for PK assessments will be collected for all subjects on Baseline/Day 1 and Weeks 1, 2, and 4.

- Baseline/Day 1 - sample should be collected 2 (±1) hrs postdose.
- Weeks 1 and 4 - samples should be collected at 24 (±4) hrs after previous dose (prior to dosing on visit day).
- Week 2 - samples should be collected at 24 (±4) hrs after prior dose (prior to dosing on visit day) AND at 2 (±1) hrs postdose.

If a subject requires hemodialysis or hemoperfusion therapy, a PK sample should be collected prior to initiation of the procedure (within 30 minutes), upon completion of the procedure (within 15 minutes), and approximately 1 hr after completion of the procedure. For subjects who initiate dialysis, GS-4997/PTM should be administered following dialysis on days of the procedure and at the usual time on non-dialysis days. The timing of prednisolone dosing should remain unchanged in subjects on dialysis. Additionally, a sample of dialysate should be collected at the end of the procedure and total volume of dialysate recorded. PK sampling only needs to be performed on one occasion of hemodialysis/hemoperfusion.

Note: If a subject discontinues treatment, but remains on study, PK samples do not need to be collected after the end of treatment visit.

6.4.2. Intensive PK Substudy

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6.4.3.

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6.5. Post-Treatment Assessments

6.5.1. Week 6 (± 3 Days)

Subjects will return for Follow-Up Visits starting at Week 6 after the Week 4 / End of Treatment Visit or the last dose of study drug to undergo the following:

During the Week 6 post treatment visit, the following will be performed and documented:

- Assess alcohol consumption
- Complete physical examination including vital signs
- Assessments for hepatic encephalopathy, ascites, HRS and infection
- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit

6.5.2. Weeks 8, 16, and 20 / Post Treatment Visits (± 5 Days)

During the Week 8, 16, and 20 post treatment visits, the following will be performed and documented:

- Assess alcohol consumption
- Complete physical examination including, vital signs
- Perform standard 12-lead ECG (Week 8 only)
- Assessments for hepatic encephalopathy, ascites, HRS and infection

- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
  - Blood for Biomarkers (Week 8 only)
- Urine pregnancy test (for females of childbearing potential)
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit

6.5.3. Weeks 12 and 24 / Post Treatment Visits (± 5 Days)

Subjects should be instructed to fast (no food or drink, except water), starting from midnight (00:00) or earlier, as appropriate, on the evening prior to the Week 12 and Week 24 visits to ensure an approximate 8-hour fast prior to the fasted blood sample collection the next morning.

Subjects that have their scheduled Week 12 or Week 24 visit prior to Day 84 or Day 168, will require a phone contact between Day 84-91 or Day 168-175 respectively, to assess for mortality and liver transplantation.

During the Week 12 and Week 24 post treatment visits, the following will be performed and documented:

- QoL Questionnaire (CLDQ, week 24 only)
- Assess alcohol consumption
- Complete physical examination including, vital signs, and body weight
- Assessments for hepatic encephalopathy, ascites, HRS and infection

- Obtain blood samples for:
  - Hematology
  - Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)
  - Coagulation Tests: PT, PTT, and INR
  - Blood for Biomarkers
  - ELF™ Test and LOXL2
  - Blood glucose and insulin (fasting)
  - Hemoglobin A1c
  - Lipid Profile (fasting)
  - PPD [redacted]
- Stool for microbiome testing (week 24 only)
- Urine pregnancy test (for females of childbearing potential)
- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit

6.6. Unscheduled Visits

Additional unscheduled assessments may be performed at the discretion of the investigator. At a minimum, the following will be performed and documented.

- Record all concomitant medications that the subject has taken since the previous visit
- Record any serious adverse events and all adverse events occurring since the previous visit

- Obtain blood samples for:

  — Hematology

  — Chemistry: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT)

6.7. Assessments for Premature Discontinuation from Study

Discontinuation from study drug dosing and discontinuation from the study (including follow-up visits and phone contact at week 12 and week 24) are 2 distinct actions.

Subjects with histologically confirmed AH who discontinue study drug (for any reason other than death) prior to completion of the assigned dosing period, should complete an EoT visit and all required follow-up study visits.

If biopsy results are not consistent with AH, the subject must stop study drugs within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible and have a Follow Up visit 30 days after their last dose of study drug, and be withdrawn from the study.

Subjects are considered to have completed the study after the Follow up Week 24 visit, regardless of treatment duration and/or early termination from study drug.

6.8. Criteria for Discontinuation of Study Treatment

Study medication may be discontinued in the following instances:

- Liver biopsy inconsistent with diagnosis of alcoholic hepatitis
- Subject who develops a serious adverse event consisting of a serious allergic reaction to the study drug
- Intercurrent illness that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree. Following resolution of intercurrent illness, the subject may resume study dosing at the discretion of the investigator after discussion with the Medical Monitor
- Unacceptable toxicity, or toxicity that, in the judgment of the investigator, compromises the ability to continue study-specific procedures or is considered to not be in the subject's best interest
- Subject request to discontinue for any reason

- Subject noncompliance
- Pregnancy during the study; refer to Appendix 3
- Sponsor discretion
- Discontinuation of the study at the request of Gilead, a regulatory agency or an institutional review board or independent ethics committee (IRB/IEC/EC)

6.9. Description of Assessments

6.9.1. Liver Biopsy

A core liver biopsy will be performed at Screening. Liver biopsy can be performed up to 7 days post Baseline/Day 1 visit and either via the percutaneous or transjugular route. Subjects may start treatment prior to receipt of liver biopsy result if subject has met all other eligibility assessments. In such cases, if a diagnosis consistent with AH is not confirmed on the liver biopsy, the subject must stop study drugs within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible, have a Follow Up visit 30 days later, and be withdrawn from the study.

Liver biopsy obtained during the current hospital admission prior to screening is acceptable.

6.9.2. Electrocardiogram

Standard 12-lead electrocardiogram (ECG) assessments will be performed. The Investigator will review the ECGs for any clinically significant abnormalities to ensure subject safety. Abnormal ECG findings that are considered clinically significant by the Investigator and meet the definition of an AE should be reported and recorded on the AE.

6.9.3. AUDIT / SADQ Questionnaires

The Alcohol Use Disorders Identification Test (AUDIT) is a 10-question test to determine if the subject may be at risk for alcohol abuse problems.

The Severity of Alcohol Dependence Questionnaire (SADQ) is a 20-question test to measure the presence of alcohol dependence.

AUDIT and SADQ will be completed by subjects at the screening visit. A Legally Authorized Representative may assist the subject in completing the questionnaires. If the subject is unable or refuses to complete a questionnaire, this should be documented on the relevant eCRF.

6.9.4. Quality of Life (QoL) Measures

The Chronic Liver Disease Questionnaire (CLDQ) will be collected at baseline/Day 1, Week 4, and Week 24. It is recommended to administer this questionnaire prior to the clinical and laboratory assessments. Legally Authorized Representative may assist the subject in completing the questionnaires. If the subject is unable or refuses to complete a questionnaire, this should be

documented on the relevant eCRF. The CLDQ asks 29 questions related to liver disease to measure health related quality of life in subjects with chronic liver disease.

6.9.5. Hepatic Encephalopathy

The severity of hepatic encephalopathy should be graded according to the West Haven Criteria, which are based on changes of consciousness, intellectual function, and behavior.

Grade 1 - Trivial lack of awareness; euphoria or anxiety; shortened attention span; impaired performance of addition

Grade 2 - Lethargy or apathy; minimal disorientation for time or place; subtle personality change; inappropriate behavior; impaired performance of subtraction

Grade 3 - Somnolence to semistupor, but responsive to verbal stimuli; confusion; gross disorientation

Grade 4 - Coma (unresponsive to verbal or noxious stimuli)

6.9.6. Ascites

The severity of ascites should be graded according to the following modification of the International Ascites Club Criteria.

Grade 0 – No ascites detectable clinically or by ultrasound

Grade 1 – Mild ascites only detectable by ultrasound

Grade 2 – Moderate ascites evident by moderate symmetrical distension of abdomen

Grade 3 – Large or gross ascites with marked abdominal distension

6.9.7. Hepatorenal Syndrome (HRS)

The occurrence of HRS should be confirmed based on the following diagnostic criteria of the International Ascites Club. The date of diagnosis/resolution and specific therapies for HRS should be recorded.

- Cirrhosis with ascites
- Serum creatinine >133 μmol/L (1.5 mg/dL)
- No improvement of serum creatinine (decrease to a level of ≤133 μmol/L) after at least 2 days with diuretic withdrawal and volume expansion with albumin. The recommended dose of albumin is 1 g/kg of body weight per day up to a maximum of 100 g/day.
- Absence of shock

- No current or recent treatment with nephrotoxic drugs

- Absence of parenchymal renal disease as indicated by proteinuria >500 mg/day, microhematuria (>50 red blood cells per high power field) and/or abnormal renal ultrasonography.

6.9.8. Infection

The occurrence of bacterial, fungal, or viral infections should be recorded as adverse events. Relevant data to be recorded include the source of infection, date of onset/resolution, presence of fever (temperature ≥38ºC/100.4ºF), laboratory investigations (e.g. white blood cell count, cultures, chest x-ray), and anti-microbial therapy. An infection will be considered definite in subjects with clinical evidence of infection and a positive culture from a normally sterile source (with the exception of spontaneous bacterial peritonitis [SBP] as below). All other infections will be considered probable. In subjects with ascitic fluid neutrophils ≥250 cells/mm^3, definite SBP will be recorded irrespective of culture positivity.

6.10. End of Study Definition

The end of the study will be the last subject's last observation (or visit).

7. ADVERSE EVENTS AND TOXICITY MANAGEMENT

7.1. Definitions of Adverse Events, Adverse Reactions, and Serious Adverse Events

7.1.1. Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical study subject administered a medicinal product, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign, 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 specified procedures, lack of efficacy, overdose, drug abuse/misuse reports, or occupational exposure. Preexisting events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an adverse event and must be reported.
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the screening visit that do not worsen
- Situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions)
- Overdose without clinical sequelae
- Any medical condition or clinically significant laboratory abnormality with an onset date before the consent form is signed and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history CRF.

7.1.2. Serious Adverse Events

A **serious adverse event** (SAE) is defined as an event that, at any dose, results in the following:

- Death
- Life-threatening (Note: The term “life-threatening” in the definition of “serious” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.)
- In-patient hospitalization or prolongation of existing hospitalization

- Persistent or significant disability/incapacity

- A congenital anomaly/birth defect

- A medically important event or reaction: such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is reportable under expedited reporting rules. 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 hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and subject to expedited reporting requirements.

7.1.3. Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events or Serious Adverse Events

Laboratory abnormalities without clinical significance are not recorded as AEs or SAEs. However, laboratory abnormalities (eg, clinical chemistry, hematology, and urinalysis) that require medical or surgical intervention or lead to IMP interruption, modification, or discontinuation must be recorded as an AE, as well as an SAE, if applicable. In addition, laboratory or other abnormal assessments (eg, electrocardiogram, x-rays, vital signs) that are associated with signs and/or symptoms must be recorded as an AE or SAE if they meet the definition of an AE or SAE as described in Sections 7.1.1 and 7.1.2. If the laboratory abnormality is part of a syndrome, record the syndrome or diagnosis (eg, anemia), not the laboratory result (ie, decreased hemoglobin).

7.2. Assessment of Adverse Events and Serious Adverse Events

The investigator or qualified subinvestigator is responsible for assessing AEs and SAEs for causality and severity, and for final review and confirmation of accuracy of event information and assessments.

7.2.1. Assessment of Causality for Study Drugs and Procedures

The investigator or qualified subinvestigator is responsible for assessing the relationship to IMP therapy using clinical judgment and the following considerations:

- **No**: Evidence exists that the adverse event has an etiology other than the IMP. For SAEs, an alternative causality must be provided (eg, pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

- **Yes**: There is reasonable possibility that the event may have been caused by the investigational medicinal product.

It should be emphasized that ineffective treatment should not be considered as causally related in the context of adverse event reporting.

The relationship to study procedures (eg, invasive procedures such as venipuncture or biopsy) should be assessed using the following considerations:

- **No:** Evidence exists that the adverse event has an etiology other than the study procedure.
- **Yes:** The adverse event occurred as a result of protocol procedures, (eg, venipuncture)

7.2.2. Assessment of Severity

The severity grading of AEs will be assessed as Grade 1, 2, 3, or 4 according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, which can be found at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf.

For AEs associated with laboratory abnormalities, the event should be graded on the basis of the clinical severity in the context of the underlying conditions; this may or may not be in agreement with the grading of the laboratory abnormality.

The distinction between the seriousness and the severity of an adverse event should be noted. Severe is a measure of intensity; thus, a severe reaction is not necessarily a serious reaction. For example, a headache may be severe in intensity, but would not be classified as serious unless it met one of the criteria for serious events listed above.

Investigator Requirements and Instructions for Reporting Adverse Events and Serious Adverse Events to Gilead

Requirements for collection prior to study drug initiation:

After informed consent, but prior to initiation of study medication, the following types of events should be reported on the case report form (eCRF): all SAEs and adverse events related to protocol-mandated procedures.

Adverse Events

Following initiation of study medication, collect all AEs, regardless of cause or relationship, until 30 days after last administration of study IMP must be reported to the eCRF database as instructed.

All AEs should be followed up until resolution or until the adverse event is stable, if possible. Gilead Sciences may request that certain AEs be followed beyond the protocol defined follow up period.

Serious Adverse Events

All SAEs, regardless of cause or relationship, that occurs after the subject first consents to participate in the study (ie, signing the informed consent) and throughout the duration of the study, including the protocol-required post treatment follow-up period, must be reported to the eCRF database and Gilead Drug Safety and Public Health (DSPH) as instructed. This also includes any SAEs resulting from protocol-associated procedures performed after informed consent is signed.

Any SAEs and deaths that occur after the post treatment follow-up visit but within 30 days of the last dose of study IMP, regardless of causality, should also be reported.

Investigators are not obligated to actively seek SAEs after the protocol defined follow up period however; if the investigator learns of any SAEs that occur after study participation has concluded and the event is deemed relevant to the use of IMP, he/she should promptly document and report the event to Gilead DSPH.

- All AEs and SAEs will be recorded in the eCRF database within the timelines outlined in the eCRF completion guideline.

- Electronic Serious Adverse Event (eSAE) Reporting Process

- Site personnel record all SAE data in the eCRF database and from there transmit the SAE information to Gilead DSPH within 24 hours of the investigator's knowledge of the event. Detailed instructions can be found in the eCRF completion guidelines.

- If for any reason it is not possible to record the SAE information electronically, ie, the eCRF database is not functioning, record the SAE on the paper serious adverse event reporting form and submit within 24 hours to:

  Gilead Sciences DSPH Fax: +1 (650) 522-5477
  E-mail: Safety_FC@gilead.com

- As soon as it is possible to do so, any SAE reported via paper must be transcribed into the eCRF Database according to instructions in the eCRF completion guidelines.

- If an SAE has been reported via a paper form because the eCRF database has been locked, no further action is necessary.

- For fatal or life-threatening events, copies of hospital case reports, autopsy reports, and other documents are also to be submitted by e-mail or fax when requested and applicable. Transmission of such documents should occur without personal subject identification, maintaining the traceability of a document to the subject identifiers.

- Additional information may be requested to ensure the timely completion of accurate safety reports.

- Any medications necessary for treatment of the SAE must be recorded onto the concomitant medication section of the subject's eCRF and the event description section of the SAE form.

7.3. Gilead Reporting Requirements

Depending on relevant local legislation or regulations, including the applicable US FDA Code of Federal Regulations, the EU Clinical Trials Directive (2001/20/EC) and relevant updates, and other country-specific legislation or regulations, Gilead may be required to expedite to worldwide regulatory agencies reports of SAEs, serious adverse drug reactions (SADRs), or suspected unexpected serious adverse reactions (SUSARs). In accordance with the EU Clinical Trials Directive (2001/20/EC), Gilead or a specified designee will notify worldwide regulatory agencies and the relevant IEC in concerned Member States of applicable SUSARs as outlined in current regulations.

Assessment of expectedness for SAEs will be determined by Gilead using reference safety information specified in the investigator's brochure or relevant local label as applicable.

All investigators will receive a safety letter notifying them of relevant SUSAR reports associated with any study IMP. The investigator should notify the IRB or IEC of SUSAR reports as soon as is practical, where this is required by local regulatory agencies, and in accordance with the local institutional policy.

7.4. Toxicity Management

Subjects who meet any of the following laboratory criteria must stop GS-4997/placebo

- Total bilirubin > 3x Baseline/Day 1 (confirmed by on repeat testing)

- ALT and/or AST > 5x Baseline/Day1 or nadir (confirmed by on repeat testing)

- Any Grade 4 AE assessed as related to GS-4997

Treatment-emergent toxicities will be noted by the Investigator and brought to the attention of the Medical Monitor. Whether or not considered treatment-related, all subjects experiencing AEs must be monitored periodically until symptoms subside, any abnormal laboratory values have resolved or returned to baseline levels or they are considered irreversible, or until there is a satisfactory explanation for the changes observed.

- Other than in the case of the liver enzymes noted above, Grade 3 or 4 clinically significant laboratory abnormalities should be confirmed by repeat testing as soon as practical to do so (Medical monitor needs to be notified), and preferably within 3 calendar days of receipt of the original test results.

- Clinical events and clinically significant laboratory abnormalities will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03, which can be found at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

- When restarting investigational medicinal product following resolution of the adverse event, the investigational medicinal product should be restarted at full dose following discussion with the Medical Monitor.

- Any questions regarding toxicity management should be directed to the Medical Monitor.

7.5. Special Situations Reports

7.5.1. Definitions of Special Situations

Special situation reports include all reports of medication error, abuse, misuse, overdose, reports of adverse events associated with product complaints, and pregnancy reports regardless of an associated AE.

Medication error is any unintentional error in the prescribing, dispensing, or administration of a medicinal product while in the control of the health care provider, subject, or consumer.

Abuse is defined as persistent or sporadic intentional excessive use of a medicinal product by a subject.

Misuse is defined as any intentional and inappropriate use of a medicinal product that is not in accordance with the protocol instructions or the local prescribing information.

An overdose is defined as an accidental or intentional administration of a quantity of a medicinal product given per administration or cumulatively which is above the maximum recommended dose as per protocol or in the product labelling (as it applies to the daily dose of the subject in question). In cases of a discrepancy in drug accountability, overdose will be established only when it is clear that the subject has taken the excess dose(s). Overdose cannot be established when the subject cannot account for the discrepancy except in cases in which the investigator has reason to suspect that the subject has taken the additional dose(s).

Product complaint is defined as complaints arising from potential deviations in the manufacture, packaging, or distribution of the medicinal product.

7.5.2. Instructions for Reporting Special Situations

7.5.2.1. Instructions for Reporting Pregnancies

The investigator should report pregnancies in female study subjects that are identified after initiation of study medication and throughout the study, including the post study drug follow-up period, to Gilead DSPH using the pregnancy report form within 24 hours of becoming aware of the pregnancy.

Refer to Section 7.3 and the eCRF completion guidelines for full instructions on the mechanism of pregnancy reporting.

The pregnancy itself is not considered an AE nor is an induced elective abortion to terminate a pregnancy without medical reasons.

Any premature termination of pregnancy (eg, a spontaneous abortion, an induced therapeutic abortion due to complications or other medical reasons) must be reported within 24 hours as an SAE. The underlying medical reason for this procedure should be recorded as the AE term.

A spontaneous abortion is always considered to be an SAE and will be reported as described in Sections 7.1.1 and 7.1.2. Furthermore, any SAE occurring as an adverse pregnancy outcome post study must be reported to Gilead DSPH.

The subject should receive appropriate monitoring and care until the conclusion of the pregnancy. The outcome should be reported to Gilead DSPH using the pregnancy outcome report form. If the end of the pregnancy occurs after the study has been completed, the outcome should be reported directly to Gilead DSPH. Gilead DSPH contact information is as follows: Email: Safety_FC@gilead.com and Fax: +1 (650) 522-5477.

Pregnancies of female partners of male study subjects exposed to Gilead or other study drugs must also be reported and relevant information should be submitted to Gilead DSPH using the pregnancy and pregnancy outcome forms within 24 hours. Monitoring of the subject should continue until the conclusion of the pregnancy. If the end of the pregnancy occurs after the study has been completed, the outcome should be reported directly to Gilead DSPH, fax number +1 650 522-5477 or email Safety_FC@gilead.com.

Refer to Appendix 3 for Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements.

7.5.2.2. Reporting Other Special Situations

All other special situation reports must be reported on the special situations report form and forwarded to Gilead DSPH within 24 hours of the investigator becoming aware of the situation. These reports must consist of situations that involve study IMP and/or Gilead concomitant medications, but do not apply to non-Gilead concomitant medications.

Special situations involving non-Gilead concomitant medications do not need to be reported on the special situations report form; however, for special situations that result in AEs due to a non-Gilead concomitant medication, the AE should be reported on the AE form.

Any inappropriate use of concomitant medications prohibited by this protocol should not be reported as "misuse," but may be more appropriately documented as a protocol deviation.

Refer to Section 7.3 and the eCRF completion guidelines for full instructions on the mechanism of special situations reporting.

All clinical sequelae in relation to these special situation reports will be reported as AEs or SAEs at the same time using the AE eCRF and/or the SAE report form. Details of the symptoms and signs, clinical management, and outcome will be reported, when available.

8. STATISTICAL CONSIDERATIONS

8.1. Analysis Objectives and Endpoints

The objectives of the study are described in Section 2 of the protocol, and endpoints for the study are described below.

8.1.1. Primary Endpoint

The primary endpoint is the safety of GS-4997 in combination with prednisolone versus prednisolone alone in subjects with severe AH. Safety and tolerability will be evaluated by examining the incidence of treatment emergent adverse events, including serious adverse events, clinical laboratory tests, ECGs, and vital signs. The number and percentage of subjects who prematurely discontinue study drug due to adverse events will be provided. Adverse events that led to premature study drug discontinuation will be summarized by system organ class and preferred term.

8.2. Secondary Endpoints

Clinical Outcomes:

- Number (%) of subjects who die by Study Day 28, Week 12, and Week 24
- Number (%) of subjects receiving a liver transplant
- Number (%) of subjects with HRS
- Number (%) of subjects with infection
- Length of hospital stay

Measures of Hepatic Synthetic Function:

- Change from baseline in liver biochemistry tests (ALT, AST, GGT, alkaline phosphatase, bilirubin, albumin and INR) by visit
- Lille response (score <0.45) at Day 7, Lille score at Day 7 as a continuous variable, and combined score including Lille score at Day 7 and baseline MELD score
- Change from baseline in prognostic indices (MELD, CPT, and Maddrey's DF scores) by visit
- [redacted] PPD [redacted]

8.2.1. Exploratory Endpoints

PPD

8.3. Analysis Conventions

8.3.1. Analysis Sets

8.3.1.1. Efficacy

The primary analysis set for efficacy analyses will be the Full Analysis Set (FAS) which includes all subjects with severe alcoholic hepatitis who were randomized into the study and received at least one dose of study drug.

Subjects who receive study drug other than that to which they were assigned will be analyzed according to the treatment group to which they were randomized.

8.3.1.2. Safety

The primary analysis set for safety analyses will be the Safety Analysis set which includes all subjects who received at least one dose of study drug. Treatment-emergent data will be analyzed and defined as data collected from the first dose of study drug through the date of last dose of study drug plus 30 days. Subjects who received study drug other than that to which they were assigned will be analyzed according to the study drug received.

8.3.1.3. Pharmacokinetics

The Pharmacokinetic (PK) Analysis Set includes all subjects who have been administered at least one dose of active study drug (GS-4997) and have at least one non-missing postdose concentration value for the corresponding analyte in plasma or serum.

PPD

8.3.1.4. Exploratory Biomarkers

PPD

8.4. Data Handling Conventions

Missing data can have an impact on the interpretation of the trial data. In general, values for missing data will not be imputed.

Where appropriate, safety data for subjects who received study drug but did not complete the study will be included in summary statistics. For example, if a subject received study medication, the subject will be included in a summary of adverse events according to the treatment received; however, if the subject is not dosed then they will be excluded from the summary. If safety laboratory results for a subject are missing for any reason at a time point, the subject will be excluded from the calculation of summary statistics for that time point. If the subject is missing a predose value, then the subject will be excluded from the calculation of summary statistics for the predose value and the change from predose values.

Values for missing safety laboratory data, ECGs, and vital signs will not be imputed; however, a missing baseline result will be replaced with a screening result, if available. If no pre-treatment laboratory value is available, the baseline value will be assumed to be normal (ie, no grade [Grade 0]) for the summary of graded laboratory abnormalities.

8.5. Demographic Data and Baseline Characteristics

Demographic and baseline measurements will be summarized using standard descriptive methods by treatment group. Demographic summaries will include age, sex, race, and ethnicity.

Baseline characteristics summary will include body weight, height, body mass index, baseline MELD, CPT, Maddrey DF, and other baseline disease characteristic variables of interest.

8.6. Interim Analysis

An interim analysis will be conducted after all subjects have been followed through the Day 28 visit for purposes of internal decision making. The senior and executive VPs, and project lead from Clinical Research and the statistician responsible for overseeing the Liver Disease Therapeutic area will be unblinded for this interim. None of the persons unblinded will be responsible for the conduct of the study.

8.7. Efficacy Analysis

The biological activity of the combination of GS-4997 and prednisolone and prednisolone alone will be evaluated using clinical outcomes (mortality, hepatorenal syndrome [HRS], infection, liver transplantation, and length of hospital stay) and measures of hepatic function (Lille response at Day 7, and change from baseline in MELD, CPT, and Maddrey's DF scores, PPD The percentage of subjects with Lille score response (defined as a Lille score <0.45 at Day 7), and percentage of subjects who have died by Day 28 will be compared between treatment groups using a 2-sided Fisher's exact test. Time to death/liver transplantation up to Day 28, Week 12 and Week 24 will be analyzed using Kaplan-Meier methods. For continuous variables, an 8-number summary will be provided by treatment group. For categorical variables, descriptive statistics (count and percentage of subjects in each category) will be presented by treatment group. Point estimates (and when appropriate 95% CI) will be calculated for each parameter.

8.7.1. Exploratory Analysis

PPD

8.8. Safety Analysis

Safety will be evaluated by assessment of clinical laboratory tests, physical examinations, ECGs, and vital signs measurements at various time points during the study, and by the documentation of AEs.

All safety data collected while "on treatment" (ie, on or after the first dose of study drug administration up to 30 days after the last dose of study drug) will be summarized by treatment

group according to the study drug received. Safety endpoints will be analyzed as the number and percentage of subjects with events or abnormalities for categorical variables. Continuous variables will be descriptively summarized (n, mean, standard deviation, median, Q1, Q3, minimum and maximum).

8.8.1. Extent of Exposure

A subject's extent of exposure to study drug will be generated from the study drug administration page of the eCRF. Exposure data will be summarized by treatment group.

8.8.2. Adverse Events

Clinical and laboratory adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). System Organ Class (SOC), High-Level Group Term (HLGT), High-Level Term (HLT), Preferred Term (PT), and Lower-Level Term (LLT) will be attached to the clinical database. Adverse event severity will be graded using the CTCAE Version 4.03.

Events will be summarized on the basis of the date of onset for the event. Treatment-emergent adverse events (TEAEs) are defined as 1 or both of the following:

- Any AEs with an onset date on or after the study drug start date and no later than 30 days after permanent discontinuation of study drug.
- Any AEs leading to premature discontinuation of study drug.

Summaries (number and percentage of subjects) of TEAEs by SOC and PT will be provided. Treatment-emergent AEs will also be summarized by relationship to study drug and by severity. In addition, TEAEs leading to premature discontinuation of study drug and study will be summarized and listed.

All AEs collected during the course of the study will be presented in data listings with a field for treatment-emergent event (yes/no).

8.8.3. Laboratory Evaluations

Selected laboratory data (using conventional units) will be summarized using only observed data. The value and change from baseline at all scheduled time points will be summarized.

Graded laboratory abnormalities will be defined using the grading scheme in the CTCAE Version 4.03.

Incidence of treatment-emergent grade 3 or higher laboratory abnormalities, defined as most severe grade 3 or higher postbaseline value that has increased by at least one toxicity grade from baseline at any time post baseline up to 30 days after the last dose of study drug, will be summarized by treatment group. If baseline data are missing, then any graded abnormality (ie, at least a Grade 1) will be considered treatment emergent.

8.8.4. Other Safety Evaluations

Summary statistics for the 12-lead ECGs performed will be generated by analysis visit.

8.9. Pharmacokinetic Analysis

GS-4997 and GS-607509 plasma/serum concentrations in all subjects will be listed and summarized. PPD

8.9.1. Other Exploratory Analyses

PPD

.

8.10. Sample Size

The number of subjects was chosen based on clinical experience with other similar proof of concept studies. PPD

8.11. Data Monitoring Committee

An independent, external Data Monitoring Committee (DMC) that includes two hepatologists and a PhD statistician will convene once 20 subjects have been treated for 28 days and every 3 months thereafter to monitor the study for safety events including SAEs, death, grade 3 and higher AEs and grade 3 and higher treatment-emergent laboratory abnormalities.

The DMC will provide recommendations to Gilead on whether the nature, frequency, and severity of adverse effects associated with study treatment warrant the early termination of the study in the best interest of the participants, whether the study should continue as planned, or the study should continue with modifications. The DMC may also provide recommendations as needed regarding study design.

The DMC's specific activities will be defined by a mutually agreed charter, which will define the DMC's membership, conduct and meeting schedule.

While the DMC will be asked to advise Gilead regarding future conduct of the study, including possible early study termination, Gilead retains final decision-making authority on all aspects of the study.

9. RESPONSIBILITIES

9.1. Investigator Responsibilities

9.1.1. Good Clinical Practice

The investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki, International Conference on Harmonisation (ICH) guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject. These standards are consistent with the European Union Clinical Trials Directive 2001/20/EC and Good Clinical Practice Directive 2005/28/EC.

The investigator will ensure adherence to the basic principles of Good Clinical Practice, as outlined in 21 CFR 312, subpart D, "Responsibilities of Sponsors and Investigators," 21 CFR, part 50, 1998, and 21 CFR, part 56, 1998.

The investigator and all applicable subinvestigators will comply with 21 CFR, Part 54, 1998, providing documentation of their financial interest or arrangements with Gilead, or proprietary interests in the investigational drug under study. This documentation must be provided prior to the investigator's (and any subinvestigator's) participation in the study. The investigator and subinvestigator agree to notify Gilead of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date when the last subject completes the protocol-defined activities.

9.1.2. Institutional Review Board (IRB)/Independent Ethics Committee (IEC)/Ethics Committee (EC) Review and Approval

The investigator (or sponsor as appropriate according to local regulations) will submit this protocol, informed consent form, and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) to an IRB/IEC/EC. The investigator will not begin any study subject activities until approval from the IRB/IEC/EC and regulatory agency, if required, has been documented and provided as a letter to the investigator.

Before implementation, the investigator will submit to and receive documented approval from the IRB/IEC/EC for any modifications made to the protocol or any accompanying material to be provided to the subject after initial approval, with the exception of those necessary to reduce immediate risk to study subjects.

9.1.3. Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and before undertaking any study-related procedures. The investigator must use the most current IRB/IEC/EC approved consent form for documenting

written informed consent. Each informed consent (or assent as applicable) will be appropriately signed and dated by the subject or the subject's legally authorized representative and the person conducting the consent discussion, and also by an impartial witness if required by IRB/IEC/EC. The consent form will inform subjects about genomic testing and sample retention, and their right to receive clinically relevant genomic analysis results.

9.1.4. Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject initials, date of birth, another unique identifier (as allowed by local law) and an identification code will be recorded on any form or biological sample submitted to the sponsor, IRB/IEC/EC, or laboratory. Laboratory specimens must be labeled in such a way as to protect subject identity while allowing the results to be recorded to the proper subject. Refer to specific laboratory instructions for further details. NOTE: The investigator must keep a screening log showing codes, names, and addresses for all subjects screened and for all subjects enrolled in the trial. Subject data will be processed in accordance with all applicable regulations.

The investigator agrees that all information received from Gilead, including but not limited to the investigator brochure, this protocol, eCRF, the IMP, and any other study information, remain the sole and exclusive property of Gilead during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Gilead. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

9.1.5. Study Files and Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) investigator's study file, and (2) subject clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IRB/IEC/EC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

The required source data should include sequential notes containing at least the following information for each subject:

- Subject identification (name, date of birth, gender);
- Documentation that subject meets eligibility criteria, ie, history, physical examination, and confirmation of diagnosis (to support inclusion and exclusion criteria);

- Documentation of the reason(s) a consented subject is not enrolled;
- Participation in study (including study number);
- Study discussed and date of informed consent;
- Dates of all visits;
- Documentation that protocol specific procedures were performed;
- Results of efficacy parameters, as required by the protocol;
- Start and end date (including dose regimen) of IMP, including dates of dispensing and return;
- Record of all adverse events and other safety parameters (start and end date, and including causality and severity);
- Concomitant medication (including start and end date, dose if relevant; dose changes);
- Date of study completion and reason for early discontinuation, if it occurs.

All clinical study documents must be retained by the investigator until at least 2 years or according to local laws, whichever is longer, after the last approval of a marketing application in an ICH region (ie, United States, Europe, or Japan) and until there are no pending or planned marketing applications in an ICH region; or, if no application is filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and regulatory authorities have been notified. Investigators may be required to retain documents longer if specified by regulatory requirements, by local regulations, or by an agreement with Gilead. The investigator must notify Gilead before destroying any clinical study records.

Should the investigator wish to assign the study records to another party or move them to another location, Gilead must be notified in advance.

If the investigator cannot provide for this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and Gilead to store these records securely away from the site so that they can be returned sealed to the investigator in case of an inspection. When source documents are required for the continued care of the subject, appropriate copies should be made for storage away from the site.

9.1.6. Electronic Case Report Forms

For each subject consented, an eCRF will be completed by an authorized study staff member whose training for this function is documented according to study procedures. The eCRF should be completed in a timely manner to enable the sponsor to perform central monitoring of safety data. Subsequent to data entry, a study monitor will perform source data verification within the EDC system. Original entries as well as any changes to data fields will be stored in the audit

trail of the system. Prior to database lock (or any interim time points as described in the clinical data management plan), the investigator will use his/her log in credentials to confirm that the forms have been reviewed, and that the entries accurately reflect the information in the source documents. The eCRF capture the data required per the protocol schedule of events and procedures. System-generated or manual queries will be issued to the investigative site staff as data discrepancies are identified by the monitor or internal Gilead staff, who routinely review the data for completeness, correctness, and consistency. The site coordinator is responsible for responding to the queries in a timely manner, within the system, either by confirming the data as correct or updating the original entry, and providing the reason for the update (eg, data entry error). At the conclusion of the trial, Gilead will provide the site with a read-only archive copy of the data entered by that site. This archive must be stored in accordance with the records retention requirements outlined in Section 9.1.5.

9.1.7. Investigational Medicinal Product Accountability and Return

The investigator or designee (ie, pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgment of receipt of each shipment of study product (quantity and condition), subject dispensing records, and returned or destroyed study product. Dispensing records will document quantities received from Gilead Sciences and quantities dispensed to subjects, including lot number, date dispensed, subject identifier number, subject initials, and the initials of the person dispensing the medication.

At study initiation, the monitor will evaluate the site's standard operating procedure of investigational medicinal product/destruction in order to ensure that it complies with Gilead Sciences requirements. Drug may be returned or destroyed on an ongoing basis during the study, if appropriate. At the end of the study, following final drug inventory reconciliation by the monitor, the study site will dispose of and/or destroy all unused investigational medicinal product supplies, including empty containers, according to these procedures. If the site cannot meet Gilead Science's requirements for disposal, arrangements will be made between the site and Gilead Sciences or its representative for destruction or return of unused investigational medicinal product supplies.

All drug supplies and associated documentation will be periodically reviewed and verified by the study monitor over the course of the study.

9.1.8. Inspections

The investigator should understand that source documents for this trial should be made available to appropriately qualified personnel from Gilead Sciences and its representatives, to IRBs/IECs/ECs, or to regulatory authority or health authority inspectors.

9.1.9. Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

9.2. Sponsor Responsibilities

9.2.1. Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by Gilead. The investigator must submit all protocol modifications to the IRB/IEC/EC in accordance with local requirements and receive documented approval before modifications can be implemented.

9.2.2. Study Report and Publications

A clinical study report (CSR) will be prepared and provided to the regulatory agency. Gilead will ensure that the report meets the standards set out in the ICH Guideline for Structure and Content of Clinical Study Reports (ICH E3). Note that an abbreviated report may be prepared in certain cases.

Investigators in this study may communicate, orally present, or publish in scientific journals or other scholarly media only after the following conditions have been met:

- The results of the study in their entirety have been publicly disclosed by or with the consent of Gilead in an abstract, manuscript, or presentation form; or
- The study has been completed at all study sites for at least 2 years

No such communication, presentation, or publication will include Gilead's confidential information (see Section 9.1.4).

The investigator will submit to Gilead any proposed publication or presentation along with the respective scientific journal or presentation forum at least 30 days before submission of the publication or presentation. The investigator will comply with Gilead's request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days in order to obtain patent protection if deemed necessary.

9.3. Joint Investigator/Sponsor Responsibilities

9.3.1. Payment Reporting

Investigators and their study staff may be asked to provide services performed under this protocol, eg, attendance at Investigator's Meetings. If required under the applicable statutory and regulatory requirements, Gilead will capture and disclose to Federal and State agencies any expenses paid or reimbursed for such services, including any clinical trial payments, meal, travel expenses or reimbursements, consulting fees, and any other transfer of value.

9.3.2. Access to Information for Monitoring

In accordance with regulations and guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the accuracy of the data recorded in the eCRF.

The monitor is responsible for routine review of the eCRF at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The monitor should have access to any subject records (including electronic medical records, if applicable) needed to verify the entries on the eCRF. The investigator agrees to cooperate with the monitor to ensure that any problems detected through any type of monitoring (central, on site) are resolved.

9.3.3. Monitoring and Oversight of Biomarker Specimens

Biomarker research specimens will be tracked in a manner consistent with Good Clinical Practice by a quality-controlled, auditable, and appropriately validated laboratory information management system, to ensure compliance with data confidentiality as well as adherence to authorized use of specimens as specified in this protocol and in the Informed Consent Form.

9.3.4. Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Gilead may conduct inspections or audits of the clinical study. If the investigator is notified of an inspection by a regulatory authority the investigator agrees to notify the Gilead medical monitor immediately. The investigator agrees to provide to representatives of a regulatory agency or Gilead access to records, facilities, and personnel for the effective conduct of any inspection or audit.

9.3.5. Study Discontinuation

Both the sponsor and the investigator reserve the right to terminate the study at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority (ies), IRBs, IECs, and ECs. In terminating the study, Gilead and the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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11. APPENDICES

Appendix 1. Investigator Signature Page

GILEAD SCIENCES, INC.
333 LAKESIDE DRIVE
FOSTER CITY, CA 94404

STUDY ACKNOWLEDGEMENT

A Phase 2, Double-Blind, Randomized Study Evaluating the Safety, Tolerability, and Efficacy of GS-4997 in Combination with Prednisolone versus Prednisolone Alone in Subjects with Severe Alcoholic Hepatitis (AH)

GS-US-416-2124, Original, 12 April 2016

This protocol has been approved by Gilead Sciences, Inc. The following signature documents this approval.

Rob Myers

Rob Myers, MD
Medical Monitor

PPD

Apr 12, 2016

Date

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Gilead Sciences, Inc. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

Principal Investigator Name (Printed)

Signature

Date

Site Number

Appendix 2. Study Procedures Table

	Screening[a]	Treatment					Post-Treatment							
	-14 days	Baseline/ Day 1	Week 1	Week 2	Week 3	Week 4/EoT[r]	Week 6	Week 8	Week 12[s]	Week 16	Week 20	Week 24[s]	Follow Up visit[t]	Unscheduled Visit
			±3 days					±5 days						
Informed Consent	X													
Review Inclusion/Exclusion Criteria	X	X	X											
Medical History	X													
AUDIT / SADQ[b]	X													
QoL (CLDQ)[b]		X				X						X		
Alcohol Consumption	X	X	X	X	X	X	X	X	X	X	X	X		
Complete Physical Exam[c]	X	X	X	X	X	X	X	X	X	X	X	X		
Height	X													
Weight	X	X				X			X			X		
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X		
12-lead ECG[d]	X	X				X		X						
Urine Drug Screen[e]	X													
Urine Collection	X													
Hepatic Encephalopathy[c]	X	X	X	X	X	X	X	X	X	X	X	X		
Ascites[c]	X[f]	X	X	X	X	X	X	X	X	X	X	X		
HRS[d]	X	X	X	X	X	X	X	X	X	X	X	X		
Infection[d]	X	X	X	X	X	X	X	X	X	X	X	X		
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Chemistry[g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation Tests[h]	X	X	X	X	X	X	X	X	X	X	X	X		
Pregnancy Test[i]	X	X				X		X	X	X	X	X		
ELF test, LOXL2		X				X			X			X		

	Screening[a]	Treatment					Post-Treatment							Unscheduled Visit
	-14 days	Baseline/ Day 1	Week 1	Week 2	Week 3	Week 4/EoT[r]	Week 6	Week 8	Week 12[s]	Week 16	Week 20	Week 24[s]	Follow Up visit[t]	
			±3 days				±5 days							
Hepatitis B and C, HIV serology	X													
Chest X-ray	X													
Liver Ultrasound	X													
Blood Cultures	X													
Ascitic Fluid Tap[j]	X													
Liver Biopsy	X[k]													
Blood Glucose (fasting)[l]	X	X				X			X			X		
Insulin (fasting)[l]	X	X				X			X			X		
Hemoglobin A1c		X							X			X		
Lipid Profile (fasting)[l]	X	X				X			X			X		
Review Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PPD														
Sparse PK Sample[n]		X	X	X		X[n]								
Stool Collection		X				X						X		
Blood Sample for Biomarkers[o]	X	X	X	X		X		X	X			X		
Randomization		X												

	Screening[a]	Treatment					Post-Treatment							
	-14 days	Baseline/ Day 1	Week 1	Week 2	Week 3	Week 4/EoT[r]	Week 6	Week 8	Week 12[s]	Week 16	Week 20	Week 24[s]	Follow Up visit[t]	Unscheduled Visit
			±3 days				±5 days							
GS-4997 Dispensing		X												
Prednisolone Dispensing		X	X		X									
PPD														
Genomic Sample[q]		X												
Study Drug Adherence			X	X	X	X								

a The visit window may be extended under special circumstances with explicit approval of the Medical Monitor. Subjects who fail to meet eligibility criteria due to an abnormal laboratory result may undergo re-testing of the abnormal analyte once during the screening window. This will be done at the discretion of the investigator and also with prior approval of the Medical Monitor.

b A Legally Authorized Representative may assist the subject in completing the questionnaires. If the subject is unable or refuses to complete a questionnaire, this should be documented on the relevant eCRF.

c Perform a complete Physical Exam with a focus on ascites and encephalopathy.

d Please see section 6.9 for description of assessments and record diagnosis/abnormal findings as an AE.

e Drug screen for amphetamines, cocaine and opiates (ie heroin, morphine).

f Subjects with evidence of sepsis or gastrointestinal bleeding may be treated for a minimum of 2 days with appropriate antibiotics. Once deemed stable, subject may continue screening and randomized if eligible.

g Chemistry will include: alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, alkaline phosphatase, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, lactate dehydrogenase (LDH), magnesium, phosphorus, potassium, sodium, total and direct bilirubin, total protein, uric acid, gamma-glutamyl transferase (GGT).

h Coagulation Panel includes: PT, PTT and INR.

i Females of childbearing potential only. Serum pregnancy test at Screening. Urine pregnancy test for other visits.

j Paracentesis with ascitic fluid cell count, differential, and culture will be performed if clinically-detectable ascites is present.

k Liver biopsy obtained during the current hospital admission prior to screening is acceptable. Liver biopsy can be performed up to 7 days post Baseline/Day 1. However, if biopsy results are not consistent with AH, the subject will discontinue study drug within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible and attend a 30-day FU visits and be withdrawn from the study.

l Subjects must be in a fasted state for at least 8 hours prior to blood collection.

m PPD

n Please refer to Section 6.4.1 for sample collection instructions. If subject's last dose of study drug is prior to the Week 4 visit, PK sample can still be collected at Week 4 as long as last dose date and time is prior to sample collection.

o Biomarker phospho-p38 blood sample will be collected prior to the first dose of study drug. This collection will only occur at select US study sites.

p PPD

q PPD

r Subjects discontinuing the study at any time for any reason should complete the procedures required for the Week 4 / End of Treatment Visit. Additionally, subjects that discontinue treatment prior to week 4 will require a phone contact between study days 28-35 to assess for mortality and liver transplantation.

s Subjects with a Week 12 or Week 24 visit prior to Day 84 or Day 168 respectively will be required to have a telephone contact between Days 84-89 or 168-173 to assess for mortality and liver transplantation.

t Subjects whose liver biopsy results are not consistent with AH, must stop study drugs within 3 days of receipt of liver biopsy result, attend an EoT visit as soon as possible and have a Follow Up visit 30 days after their last dose of study drug, and be withdrawn from the study.

Appendix 3. Pregnancy Precautions, Definition for Female of Childbearing Potential, and Contraceptive Requirements

1) Definitions

a. Definition of Childbearing Potential

For the purposes of this study, a female born subject is considered of childbearing potential following the initiation of puberty (Tanner stage 2) until becoming post-menopausal, unless permanently sterile or with medically documented ovarian failure.

Women are considered to be in a postmenopausal state when they are ≥ 54 years of age with cessation of previously occurring menses for ≥ 12 months without an alternative cause. In addition, women of any age with amenorrhea of ≥ 12 months may also be considered postmenopausal if their follicle stimulating hormone (FSH) level is in the postmenopausal range and they are not using hormonal contraception or hormonal replacement therapy.

Permanent sterilization includes hysterectomy, bilateral oophorectomy, or bilateral salpingectomy in a female subject of any age.

b. Definition of Male Fertility

For the purposes of this study, a male born subject is considered fertile after the initiation of puberty unless permanently sterile by bilateral orchidectomy or medical documentation.

2) Contraception Requirements for Female Subjects

a. Study Drug Effects on Pregnancy and Hormonal Contraception

GS-4997 is contraindicated in pregnancy as a malformation effect is suspected, based on non-clinical data. In rats and rabbits, GS-4997 administration was associated with effects on embryo-fetal development at maternally toxic doses. This included total litter loss, increased resorptions and post implantation loss, reduced fetal weights, and visceral and skeletal malformations and variations. Embryofetal effects were observed in rats and rabbits at exposures (AUC24hr) that were 67- and 12-fold higher, respectively, than the projected exposure at the proposed human dose of 18 mg/day. The NOELs for embryofetal development in rats and rabbits were 15 and 10 mg/kg/day, respectively. The exposure margins at these doses as compared to the maximum proposed human dose are 13- and 3-fold, respectively.

Preclinical data indicate that GS-4997 is unlikely to reduce the exposure of hormonal contraceptives through induction of human drug metabolizing enzymes or drug transporters. This is supported by clinical DDI data, which demonstrated multiple doses of GS-4997 did not result in increased activity of CYP3A4 or P-gp based on plasma exposures of sensitive probe substrates (midazolam and digoxin). Similarly, preclinical and clinical data indicate GS-4997 is unlikely to cause a clinically relevant increase in the exposure of hormonal contraceptives through inhibition of drug metabolizing enzymes or drug transporters. Please refer to the latest version of the investigator's brochure for additional information.

b. Contraception Requirements for Female Subjects of Childbearing Potential

The inclusion of female subjects of childbearing potential requires the use of highly effective contraceptive measures. They must have a negative serum pregnancy test at Screening and a negative pregnancy test on the Baseline/Day 1 visit prior to randomization. Pregnancy tests will be performed at monthly intervals thereafter. Female subjects must agree to one of the following from Screening until 90 days following the end of relevant systemic exposure.

- Complete abstinence from intercourse of reproductive potential. Abstinence is an acceptable method of contraception only when it is in line with the subject's preferred and usual lifestyle.

  Or

- Consistent and correct use of 1 of the following methods of birth control listed below.

  — Intrauterine device (IUD) with a failure rate of <1% per year

  — Tubal sterilization

  — Essure micro-insert system (provided confirmation of success 3 months after procedure)

  — Vasectomy in the male partner (provided that the partner is the sole sexual partner and had confirmation of surgical success 3 months after procedure)

- The above described methods are considered preferred methods of highly effective contraception in this protocol.

  - Should female subjects wish to use a hormonally based method, use of a male condom by the female subject's male partner is required. Subjects who utilize a hormonal contraceptive as one of their birth control methods must have used the same method for at least three months prior to study dosing. Hormonally-based contraceptives permitted for use in this protocol are as follows:

    - Hormonal methods (each method *must* be used with a condom in the male partner)

      - Oral contraceptives (either combined or progesterone only)

      - Injectable progesterone

      - Implants of levonorgestrel

      - Transdermal contraceptive patch

      - Contraceptive vaginal ring

Female subjects must also refrain from egg donation and in vitro fertilization during treatment and until at least 90 days after the end of relevant systemic exposure.

3) Contraception Requirements for Male Subjects

It is theoretically possible that a relevant systemic concentration may be achieved in a female partner from exposure of the male subject's seminal fluid. Therefore, male subjects with female partners of childbearing potential must use condoms during treatment until the end of relevant systemic exposure. Female partners of male study subjects are asked to select one of the above methods.

Male subjects must also refrain from sperm donation during treatment and until at least 90 days after the end of relevant systemic exposure.

4) Unacceptable Birth Control Methods

Birth control methods that are unacceptable include periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM). Female condom and male condom should not be used together.

5) Procedures to be Followed in the Event of Pregnancy

Subjects will be instructed to notify the investigator if they become pregnant at any time during the study, or if they become pregnant within 90 days of last study drug dose. Subjects who become pregnant or who suspect that they are pregnant during the study must report the information to the investigator and discontinue study drug immediately. Subjects whose partner has become pregnant or suspects she is pregnant during the study must report the information to the investigator. Instructions for reporting pregnancy, partner pregnancy, and pregnancy outcome are outlined in Section 7.5.2.1.