Protocol No.: GSN000300 Protocol Version No.: Final 2.0; Amendment 1 Protocol Date: 10 May 2017	Compound No.: GKT137831 Sponsor: Genkyotex Author: Cmed Clinical Services

CLINICAL STUDY PROTOCOL

A Double-Blind, Randomized, Placebo-Controlled Clinical Trial to Assess the Efficacy and Safety of Oral GKT137831 in Patients with Primary Biliary Cholangitis Receiving Ursodeoxycholic Acid and with Persistently Elevated Alkaline Phosphatase

Protocol Number:	GSN000300
Test Product:	GKT137831
Indication:	Primary Biliary Cholangitis
Study/IND Sponsor:	Genkyotex
Development Phase:	Phase 2
EudraCT No.:	2016-004599-23
Sponsor Program Official:	Philippe Wiesel, MD Chief Medical Officer Genkyotex SA 516, rue Pierre et Marie Curie 31670 Labège France
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Original Protocol Version No.:	Final 1.0 dated 23 February 2017
Amendment 1:	Final 2.0 dated 10 May 2017

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SPONSOR SIGNATURE PAGE

PROTOCOL TITLE:	A Double-Blind, Randomized, Placebo-Controlled Clinical Trial to Assess the Efficacy and Safety of Oral GKT137831 in Patients with Primary Biliary Cholangitis Receiving Ursodeoxycholic Acid and with Persistently Elevated Alkaline Phosphatase
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PROTOCOL VERSION AND DATE:	Final 2.0; Amendment 1; 10 May 2017
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PROTOCOL NUMBER: GSN000300

PROTOCOL VERSION AND DATE: Final 2.0; Amendment 1; 10 May 2017

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PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial to Assess the Efficacy and Safety of Oral GKT137831 in Patients with Primary Biliary Cholangitis Receiving Ursodeoxycholic Acid and with Persistently Elevated Alkaline Phosphatase

PROTOCOL NUMBER: GSN000300

PROTOCOL VERSION AND DATE: Final 2.0; Amendment 1; 10 May 2017

INVESTIGATOR STATEMENT:

I agree to conduct the above-entitled study in accordance with the terms and conditions of this protocol, ICH GCP guidelines, the provisions of the Declaration of Helsinki and with all applicable regulatory requirements. All information pertaining to the study shall be treated in a confidential manner.

I agree to conduct the study in person or to supervise the trial.

I agree to ensure that all who assist me in the conduct of the study are aware of their obligations.

Site Investigator:

Signed: _____ Date: _____

Name:
Title:
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LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism And Excretion
AE	Adverse Event
AESI	Adverse Event of Special Interest
ALD	Alcoholic Liver Disease
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMA	Anti-Mitochondrial Antibody(ies)
ANA	Anti-Nuclear Antibody(ies)
ANCOVA	Analysis of Covariance
APRI	AST to Platelet Ratio Index
AST	Aspartate Aminotransferase
AUC	Area Under The Curve
BID	Twice Daily
BSEP	Bile Salt Export Pump
CFR	Code of Federal Regulations
CK-18	Cytokeratin-18
C_{max}	Maximum Plasma Concentration
C_{min}	Minimum Plasma Concentration
CRO	Clinical Research Organization
CSR	Clinical Study Report
CV%	Coefficient of Variation
DBP	Diastolic Blood Pressure
DILI	Drug Induced Liver Injury
DKD	Diabetic Kidney Disease
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
ELF	Enhanced Liver Fibrosis
EMA	European Medicines Agency
EudraCT	European Union Drug Regulatory Agency Clinical Trial
FDA	Food And Drug Administration
FIB-4	Fibrosis-4
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma Glutamyl Transferase
GMP	Good Manufacturing Practice
HDPE	High-Density Polyethylene
hsCRP	High Sensitivity C-Reactive Protein
HTD	Highest Tolerated Dose
IB	Investigator’s Brochure

ICF	Informed Consent Form
ICH	International Conference On Harmonization
IEC	Independent Ethics Committee
IgM	Immunoglobulin M
IIV	Inter-Individual Variability
IL	Interleukin
IMP	Investigational Medicinal Product
IND	Investigational New Drug (Application)
INR	International Normalized Ratio
IPF	Idiopathic Pulmonary Fibrosis
IRB	Institutional Review Board
ITT	Intent-To-Treat
IU	International Units
IUD	Intrauterine Device
IUS	Intrauterine Hormone-Releasing System
IWRS	Interactive Web-Based Randomization System
LREs	Liver Related Events
MAR	Missing at Random
MARS	Molecular Adsorbent Recirculation System
MCP-1	Monocytic Chemoattractant Protein-1
MELD	Model For End Stage Liver
MNAR	Missing Not at Random
MSAP	Modeling and Simulation Analysis Plan
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NASH	Nonalcoholic Steatohepatitis
NOAEL	No Observed Adverse Effect Level
OCA	Obeticholic Acid
OD	Once Daily
PBC	Primary Biliary Cholangitis
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Per Protocol
QA	Quality Assurance
QC	Quality Control
QoL	Quality of Life
QTcF	Corrected QT Interval (Fredericia's Formula)
RBC	Red Blood Cell
ROS	Reactive Oxygen Species
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SMB	Safety Monitoring Board
SOP	Standard Operating Procedure

SSc	Systemic Sclerosis
$t_{½}$	Half-Life
TEAE	Treatment-Emergent Adverse Event
t_{max}	Time To Maximum Plasma Concentration
TSH	Thyroid-Stimulating Hormone
UDCA	Ursodeoxycholic Acid
ULN	Upper Limit Of Normal
US	United States
VAS	Visual Analog Scale
WBC	White Blood Cell
WHO	World Health Organization
WOCBP	Women of Child Bearing Potential

PROTOCOL SUMMARY

Title:	A Double-Blind, Randomized, Placebo-Controlled Clinical Trial to Assess the Efficacy and Safety of Oral GKT137831 in Patients with Primary Biliary Cholangitis Receiving Ursodeoxycholic Acid and with Persistently Elevated Alkaline Phosphatase
Study Overview:	This will be a double-blind, randomized, placebo-controlled, multicenter, parallel group phase 2 trial assessing a 24-week period of treatment with oral GKT137831 administered in addition to standard of care medication (ursodeoxycholic acid; UDCA) in subjects with primary biliary cholangitis (PBC). Subjects will be assessed for their eligibility during the screening period (Visit 1), which will last up to 4 weeks, until the baseline/Day 1 visit (Visit 2). Eligible subjects will be randomized to oral GKT137831 (400 mg once daily (OD) or 400 mg twice daily (BID) or placebo, according to a 1:1:1 randomization ratio, stratified at study entry by disease severity defined as baseline serum gamma glutamyl transferase (GGT) < 2.5 x the upper normal limit (ULN) or ≥ 2.5 x the ULN. Subjects will self-administer orally 400 mg of GKT137831 OD or 400 mg of GKT137831 BID or matching placebo for a total of 24 weeks. Baseline assessments will be performed at baseline/Day 1 (Visit 2). The 24-week treatment period will include assessments after 2 weeks of treatment (Visit 3), after 6 weeks of treatment (Visit 4), after 12 weeks of treatment (Visit 5), after 18 weeks of treatment (Visit 6) and after 24 weeks of treatment (End of Treatment/Visit 7). Subjects will be followed up for 28 days after the end of treatment (Week 28/Visit 8), totaling 6 post-baseline visits. Subjects who discontinue treatment before Week 24 will have an early termination visit (premature end of treatment visit). Pharmacokinetic samples will be taken at baseline, Week 2, Week 12 and Week 18. Subjects will be taking a stable dose of UDCA at enrollment and will continue their UDCA treatment at a stable dose (no changes

	at all) during the treatment period. A Safety Monitoring Board (SMB) will oversee the conduct of the study to ensure the safety of participating subjects. An interim analysis will be conducted when approximately 90 subjects have completed their Week 6 visit.
Objectives:	**Primary:** To evaluate the efficacy of oral GKT137831 in comparison with placebo, in subjects with PBC receiving UDCA and with persistently elevated Alkaline Phosphatase (ALP). **Secondary:** • To evaluate the safety of oral GKT137831 in comparison with placebo, in subjects with PBC. • To estimate the population pharmacokinetics (PK) of GKT137831 and explore any potential Pharmacokinetics-Pharmacodynamics (PK-PD) relationships in this subject population. • To explore any relationship between genetic parameters and therapeutic responses in a subset of subjects.
Primary Efficacy Endpoint:	The percent change from baseline to Week 24 (Visit 7) in serum GGT.
Secondary Efficacy Endpoints:	• Absolute and percent change in serum GGT from baseline to each assessment. • Absolute change in Enhanced Liver Fibrosis (ELF) score from baseline to Weeks 12 and 24. • Absolute and percent change in serum ALP from baseline to each assessment. • Absolute and percent change in serum levels of high-sensitivity C-reactive protein (hsCRP) and fibrinogen, from baseline to each assessment. • Absolute and percent change in serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), conjugated and total bilirubin, from baseline to each assessment. • Absolute and percent change in the Fibrosis-4 (FIB-4) and

	AST to Platelet Ratio Index (APRI) scores, from baseline to each assessment (FIB-4: age (years) x AST (IU/L)/(platelet count (10^9/L) x (ALT (IU/L)1⁄2, APRI: AST (IU/L)/ upper normal limit AST)x100/platelet count (10^9/L). • Absolute and percent change in liver stiffness as assessed by transient elastography (FibroScan® or similar technology), from baseline to Week 24, in patients with values at baseline and Week 24. • Absolute and percent change in serum levels of collagen fragments indicative of collagen formation and degradation, from baseline to Weeks 12 and 24. • Absolute and percent change in total bile acids from baseline to Week 12 and 24.

Eligibility Criteria	Eligibility assessments will be conducted at Screening (Visit 1). When confirming eligibility at baseline/Day 1 (Visit 2), not every eligibility assessment needs to be repeated i.e., confirmatory laboratory and diagnostic tests. Subjects with adverse events (AE) at baseline may need to be withdrawn in accordance with the eligibility and withdrawal criteria.
	
	Inclusion Criteria:
	1. Male or female aged 18 to 80 years, inclusive.
	2. Willing and able to give written informed consent and to comply with the requirements of the study.
	3. PBC diagnosis as demonstrated by the presence of ≥ 2 of the following 3 diagnostic factors:
	  ○ History of elevated ALP levels (> ULN) for at least 6 months
	  ○ Positive anti-mitochondrial antibody (AMA) titer or if AMA negative or in low titer (< 1:80) PBC-specific antibodies (anti-GP210 and/or anti-SP100 and/or antibodies against the major M2 components [PDC-E2, 2-oxo-glutaric acid dehydrogenase complex])
	  ○ Liver biopsy consistent with PBC (based on historic liver biopsy), including non-suppurative, destructive cholangitis affecting mainly the interlobular and septal bile ducts.
	4. Serum ALP ≥ 1.5 x ULN.
	5. Serum GGT ≥ 1.5 x ULN.
	6. UDCA treatment for at least 6 months and stable dose for at least 3 months prior to Visit 1.

	7. Subjects being treated for pruritus with colestyramine must be on a stable dose of colestyramine for at least 8 weeks prior to baseline/Day 1 (Visit 2). Subjects must be willing and able to take colestyramine at least 2 hours before or after study medication. 8. Female subjects of childbearing potential must use a highly effective method of contraception to prevent pregnancy for 4 weeks before randomization and must agree to continue strict contraception for 90 days after last administration of investigational medicinal product (IMP). Male participants with female partners of childbearing potential must be willing to use a condom and require their partner to use an additional form of adequate contraception as approved by the Investigator. This requirement begins at the time of informed consent and ends 90 days after the last administration of IMP. Male study participants must also not donate sperm from baseline until 90 days after the last administration of IMP. **Exclusion Criteria:** 1. A positive pregnancy test or breast-feeding for female subjects. 2. Any hepatic decompensation, defined as a past or current history of hepatic encephalopathy, gastrointestinal tract bleeding due to esophageal varices, or ascites. 3. International normalized ratio (INR) > 1.2 unless subject is on anticoagulant therapy. 4. ALT > 3 x ULN. 5. Total bilirubin > 1 x ULN. 6. Planned or current plasmapheresis or other extra-corporeal treatments (e.g., molecular adsorbent recirculation system (MARS)) for treatment-refractory pruritus. 7. History of liver transplantation, current placement on a liver transplant list or current Model for End Stage Liver Disease (MELD) score ≥ 15. 8. Cirrhosis with complications, including history or presence of: spontaneous bacterial peritonitis, hepatocellular carcinoma. 9. Hepatorenal syndrome (type I or II) or Screening serum creatinine > ULN. 10. Competing etiology for liver disease (e.g., hepatitis C, active

	hepatitis B, non-alcoholic steatohepatitis (NASH), alcoholic liver disease (ALD), autoimmune hepatitis, primary sclerosing cholangitis, Gilbert's Syndrome). 11. Subjects receiving prohibited medications within 3 months of Screening (Visit 1) according to the list (a, b and c) provided in Section 6.6.2. 12. Treatment with any investigational agent within 4 weeks of Visit 1 or 5 half-lives of the investigational medicinal product (whichever is longer). 13. A history of long QT syndrome. 14. Evidence of any of the following cardiac conduction abnormalities during the screening period: • A QTc Fredericia interval >450 milliseconds for males and >470 milliseconds for females. • A second or third degree atrioventricular block not successfully treated with a pacemaker. 15. History of cancer in the preceding 5 years, except adequately treated non-melanoma skin cancer, carcinoma *in situ* of the cervix, *in situ* prostate cancer, *in situ* breast ductal carcinoma, or superficial bladder cancer stage 0). 16. The occurrence of any acute infection requiring systemic antibiotic therapy within the 2 weeks prior the Screening Visit (Visit 1), or human immunodeficiency virus (HIV) infection. 17. A history of bone marrow disorder including aplastic anemia, or marked anemia defined as hemoglobin < 10.0 g/dL (or 6.2 mmol/L). 18. Any condition which, in the opinion of the Investigator, constitutes a risk or contraindication for the participation of the subject in the study, or which could interfere with the study objectives, conduct, or evaluation.
Phase:	Phase 2
Number of Sites:	Approximately 50-60 investigational sites will be initiated in North America, Europe, and Israel.
Study Duration:	Planned start: Q2 2017 Planned end: Q2 2019
Subject Participation	The total duration of double-blind treatment will be 24 weeks from

Duration:	baseline/Day 1 (Visit 2) to Week 24 (End of Treatment/Visit 7). A Screening visit (Visit 1) will be done within 4 weeks before baseline/Day 1. A follow up visit (Visit 8) will be carried out within 4 weeks after the end of treatment (i.e., Week 28).
Description of Agent or Intervention:	Capsules of GKT137831 or matching placebo to be taken orally: 400 mg OD or 400 mg BID for 24 weeks.
Estimated Time to Complete Enrollment:	7-8 months

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Primary Biliary Cholangitis

Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis, is a liver disease caused by auto-immune (T-cell-mediated) attack on the small to medium intra-lobular bile ducts [1, 2]. A continuous assault on the bile duct epithelial cells leads to their gradual destruction and eventual disappearance (i.e., ductopenia). Once immune-mediated bile duct injury has been established, the disease progresses due to chronic cholestasis, secondary inflammation and fibrosis, and may lead to liver cirrhosis and liver failure [3, 4]. The reasons for the auto-immune attack are not well understood but may involve environmental triggers in genetically susceptible individuals.

The reported prevalence of PBC ranges from 20-400 cases per million of the population with some geographical differences [5, 6]. PBC is more common in women than men (9:1 ratio) and mostly diagnosed between the ages of 30 and 60 years [7, 8].

Up to 60% of patients with PBC are asymptomatic at diagnosis and are detected through coincidental finding of abnormalities in liver biochemistry tests. The most common symptoms, where present, are fatigue and pruritus. Physical examination is often normal but, where present, common findings are: skin hyperpigmentation, jaundice, excoriations, xanthomata, xanthelasmas and, rarely, hepatosplenomegaly [9, 10].

Common laboratory test abnormalities in patients with PBC include: elevated ALP, anti-mitochondrial antibodies (AMA), anti-nuclear antibodies (ANA), and hyperlipidemia. Other findings may include elevations in serum aminotransferases and bilirubin. Complications of PBC include cirrhosis, hepatocellular carcinoma, metabolic bone disease, and malabsorption [11, 12].

PBC is often associated (co-diagnosed) with other auto-immune disorders including: Sjogren syndrome, auto-immune thyroid disease (Hashimoto's thyroiditis) and rheumatoid arthritis.

Management of patients with PBC consists of treatment of the underlying disease and management of its symptoms and complications, including: pruritus, xanthomata, hypercholesterolemia, vitamin deficiencies, anemia, malabsorption, osteoporosis, etc.

PBC is a progressive disease in most patients. It eventually becomes irreversible, and therefore untreatable. The only widely accepted treatment is UDCA. It is the only treatment recommended in guidelines issued by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver.

Although there is currently no treatment for the autoimmune injury to biliary endothelial cells, in recent years, novel therapies targeting cholestasis have improved outcomes for many patients with PBC. UDCA and more recently obeticholic acid (OCA) have been licensed by the Food and Drug Administration (FDA) and European Medicines Agency (EMA), for treatment of PBC

UDCA is a synthetic secondary bile acid, given orally at a dose of 10-15 mg/kg/day as first line treatment for PBC. The following mechanisms of action may be involved in the beneficial therapeutic effects of UDCA in PBC and other cholestatic disorders: (a) increase in the hydrophilicity of circulating bile acids; (b) stimulation of hepatocellular and ductular secretions; (c) protection against cellular injury caused by bile acids and cytokines; (d) some degree of immunomodulation and anti-inflammatory effects. Currently, treatment with UDCA represents the global standard of care [2, 3], and can delay histologic progression [4–6] and improve long-term survival [7, 8]. However, UDCA is not a uniformly effective drug, there is little benefit to symptoms, and approximately 40% of PBC patients have a sub-optimal response to UDCA [13, 14]. Patients with an inadequate response to UDCA (assessed biochemically), or those not tolerating UDCA, progress to cirrhosis and liver failure leading to liver transplant or death. Therefore, there is an unmet medical need in PBC patients with inadequate response to UDCA.

OCA is another synthetic secondary bile acid which has only recently been approved by the FDA and EMA for treatment of PBC [15, 16]. OCA is indicated for the treatment of PBC in combination with UDCA in adults with an inadequate response to UDCA, or as monotherapy in adults unable to tolerate UDCA. OCA is not yet in wide clinical use. It has a similar mechanism of action as UDCA but has been shown to achieve further reductions in ALP levels in patients with inadequate response to UDCA. This additional anti-cholestatic effect was associated with a reduction in hepatocellular damage, as shown by decreases in AST and ALT levels. However, OCA treatment achieved only small reductions in these markers of hepatocellular injury which failed to normalize to below the upper limit of the normal reference range [17]. Over time, this sustained hepatocellular injury likely contributes to progressive liver fibrosis and failure. Separately, OCA worsens the severity of pruritus, a key symptom of PBC which significantly impacts patients' quality of life. The currently available therapies mainly target cholestasis. However, there is a need for well-tolerated therapies able to address additional components of the disease, including autoimmune injury to biliary endothelial cells, bile acid-mediated hepatocellular injury, and fibrogenesis [18, 19].

2.1.2 GKT137831

GKT137831 is a small organic molecule of low molecular weight, a member of the pyrazolopyridine dione chemical class. It is a selective inhibitor of NOX 1 and 4 isoforms of the Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase family of enzymes, and is the first drug in this class of NOX inhibitors to enter the clinic.

The NADPH oxidase family (NOX) is a set of transmembrane proteins [20]. NOX enzymes require the stable assembly of transmembrane and cytosolic subunits. Upon assembly of a full enzymatic complex and activation by its substrates NADPH and molecular oxygen, NOX enzymes transport electrons through the cell membrane to produce reactive oxygen species (ROS). In turn, ROS

modulate multiple signaling pathways by oxidizing regulatory cysteine residues in target proteins. ROS can also cause other types of post-translational modification of proteins, and can target lipids and nucleic acids.

When exaggerated in duration and/or magnitude, NOX activation participates in the pathogenesis of a broad range of human diseases. In particular, the NOX1 and NOX4 isoforms have been shown to play a key role in a broad range of inflammatory and fibrotic disorders [21-25]. Importantly, recent studies have revealed that liver tissue expression of NOX1 and/or NOX4 is consistently elevated in patients with fibrotic liver diseases [26, 27].

GKT137831 is thus being investigated in several inflammatory and fibrotic disorders, including PBC, Nonalcoholic Steatohepatitis (NASH), Idiopathic Pulmonary Fibrosis (IPF), Diabetic Kidney Disease (DKD), Systemic Sclerosis (SSc). It is also under investigation in the development of the fibrotic tumor stroma.

In *in vitro* studies in isolated cells, GKT137831 was shown to attenuate signaling evoked by a number of ligands known to induce and/or drive the fibrogenic process in multiple fibrogenic pathways, including TGF-β1, PDGF, TLR4, hedgehog and angiotensin II [26]. As a result, GKT137831 markedly reduced the induction of markers of myofibroblast activation, including αSMA, fibronectin, and pro-collagen I [28, 29].

These direct anti-fibrogenic effects translate into anti-fibrotic activity in multiple *in vivo* models of liver fibrosis. Specifically, GKT137831 attenuated the development of liver fibrosis induced by experimental cholestasis (in the bile duct ligation and MDR2-/-, mouse models). GKT137831 also prevented liver fibrosis in models of NASH (in the STAM and fast food diet models [29]). GKT137831 also prevented liver fibrosis in models of NASH (in the STAM and fast food diet models [27, 30]) and in toxic hepatitis (in CCL4-induced liver injury [28]). Reduced *in vivo* fibrogenesis was associated with a marked reduction in markers of myofibroblast activation.

In addition, GKT137831 has shown potent anti-inflammatory effects in a number of biological settings, including metabolic and cholestatic liver injury. Specifically, GKT137831 prevented the induction of adhesion molecules, cytokines, and chemokines in CCL4-induced liver injury and fast food diet-induced NASH [27]. These effects on innate immunity resulted in a profound reduction of macrophage infiltration. Available data suggests that these anti-inflammatory effects are mediated through reduced activation of multiple pathways, including TLR4 and NF-κB [21]. In these studies, reduced liver inflammation was associated with a reduction in plasma levels of liver transaminases.

GKT137831 was evaluated in patients with type 2 diabetes and kidney disease. Because this patient population has a high prevalence of the metabolic syndrome and non-alcoholic fatty liver disease, the trial assessed markers of liver injury and inflammation. GKT137831 achieved statistically significant reductions in GGT and hsCRP.

These extensive pre-clinical studies and initial clinical results suggest that GKT137831 has the potential to alleviate hepatocellular injury and prevent progressive liver fibrosis.

2.1.3 Choice of Endpoints

The main objective of the trial is to evaluate the effect of GKT137831 on liver injury and inflammation, and on fibrogenesis. While plasma ALP is a reasonably well-validated surrogate endpoint of outcomes for anti-cholestatic drugs, there are no validated surrogate endpoints for anti-inflammatory and anti-fibrotic drugs. Accordingly, the primary and secondary efficacy endpoints have been selected to provide proof of concept for the therapeutic value of GKT137831, and to characterize its initial efficacy profile.

2.1.3.1 Primary Efficacy Endpoint

GGT was selected as the primary efficacy endpoint because it is consistently elevated in PBC patients, correlates with disease severity, can respond quickly to therapeutic interventions, and is likely to be modulated by GKT137831 through anti-inflammatory and redox mechanisms. In cholestatic disease, elevated GGT reflects inflammatory and cholestatic injury to bile ducts and hepatic parenchymal structures. GGT is expressed in biliary epithelial cells and hepatocytes, and is elevated in multiple biliary and non-biliary liver disorders, including PBC, PSC, NASH, ALD, and hepatitis B and C. GGT correlates with the severity of liver fibrosis across a broad range of fibrotic liver disorders [31-37]. As a result, GGT has been included in the FibroSure/FibroTest score, a non-invasive marker of liver fibrosis [38]. Importantly, GGT responds rapidly (i.e. within weeks) to the administration of UDCA, OCA, fibrates, or a modified FGF-19 [17, 39].

GGT is involved in hepatic redox homeostasis and is a marker of oxidative stress [40-42]. So, in addition to cholestatic and inflammatory injury, oxidative stress can also participate in the induction of GGT expression in PBC patients. Accordingly, GKT137831 has the potential to reduce GGT levels through anti-inflammatory and redox mechanisms in cholangiocytes and hepatocytes. While not directly relevant to PBC, the statistically significant reduction in GGT achieved with GKT137831 in patients with DKD supports this assumption. Of note, GGT levels predict renal and all-cause mortality in DKD patients [43, 44].

In summary, while GGT is not a validated surrogate endpoint of outcome in PBC, it is a clinically relevant efficacy endpoint that is well suited to demonstrate the pharmacodynamic activity of GKT137831, and together with supportive secondary endpoints, inform dose-response relationships.

2.1.3.2 Secondary Efficacy Endpoints Related to Fibrosis

The evaluation of the anti-fibrotic activity of GKT137831 is an important objective of the trial. Patients with inadequate response to UDCA undergo accelerated liver fibrosis [45]. It is therefore critical to develop novel therapies able to directly target fibrogenesis. GKT137831 has shown profound anti-fibrotic activity in multiple models of fibrotic liver diseases. It was not only effective in models of NASH, where hepatic stellate cells are the main source of activated myofibroblasts, it was also effective in cholestatic models such as MDR2-/- mice where portal fibroblasts are the source of activated myofibroblasts.

The effect of GKT137831 on liver fibrosis will be explored through the evaluation of direct non-invasive markers of fibrogenesis. These markers include the ELF score and a panel of collagen fragments. In addition, liver stiffness will be evaluated by transient elastography (performed at centers where FibroScan™ or similar equipment is available). Finally, indirect markers of fibrosis, such as FIB-4 and APRI, will be also assessed [46].

A growing body of evidence supports the use of the ELF score to evaluate anti-fibrotic therapies. The ELF score is a direct marker of fibrogenesis; its components (HA, TIMP-1, and PIIINP) have a direct role in the fibrogenic process [47-56]. The ELF score correlates well with the presence and severity of liver fibrosis in several fibrotic liver diseases, including hepatitis C, hepatitis B, NASH, PBC, autoimmune hepatitis, alcohol liver disease and others [57-62]. Furthermore, the ELF score accurately predicts disease progression in PBC, NASH, and hepatitis C, as shown by the correlation between the baseline ELF score and the occurrence of liver-related events (LREs) during follow up. Initial clinical results suggest the ELF test has the ability to detect the effect of therapeutic interventions in several fibrotic liver diseases, including PBC [19, 46, 57, 58]. In PBC patients, event-free survival was significantly lower in those with high baseline ELF. Each 1- point increase in ELF was associated with a 3-fold increase in future complications [57]. Similar data was obtained in patients with primary sclerosing cholangitis, where patients stratified by the ELF score tertiles exhibited significantly different transplant-free survival ($P < 0.001$), with higher scores associated with shorter survival, further confirmed in the validation set stratified by ELF Test tertiles ($P= 0.003$). In addition, the ELF Test distinguished between mild and severe disease as defined by clinical outcome (transplantation or death) with an area under the curve (AUC) of 0.81.

The ELF test has received the CE and has been extensively validated and reference values have been published [57, 63, 64]. Accordingly, treatment guidelines issues by NICE and EASL–EASD–EASO recommend the use of the ELF test to detect the presence of liver fibrosis and to monitor therapeutic responses.

Despite these encouraging developments, further work is required to validate the ELF score as surrogate endpoint of outcome in PBC. In particular, it is still unclear that ELF score reductions achieved by specific therapeutic interventions do predict improvements in clinical outcomes.

Importantly however, available data suggest that the ELF score will be elevated in the target patient population. In a comparable patient population, the ELF score suggested that most patients had moderate-to-severe liver fibrosis [Hirschfield, 2016]. This data is consistent with results obtained in a similar patient population in the OCA phase 3 trial [17]. In this trial, all patients had some degree of liver fibrosis, and over 50% of patients had at least septal fibrosis (OCA, FDA medical review). Therefore, the ELF score is well suited for the evaluation of anti-fibrotic effects in this trial.

2.1.3.3 Secondary Efficacy Endpoints Related to Hepatocellular Injury

Markers of hepatocellular injury such as AST or ALT are only modestly elevated in PBC patients, and have shown inconsistent responses to OCA. Accordingly, they were not selected as primary efficacy endpoints. Nevertheless, these markers, together with CK-18 and total and conjugated bilirubin will provide supportive information about drug effects on hepatocellular injury. In preclinical models of cholestatic and metabolic liver diseases, GKT137831 achieved marked reductions in liver function tests and reduced hepatocyte apoptosis.

2.1.3.4 Secondary Efficacy Endpoints Related to Immunological Processes and Inflammation

GKT137831 has shown consistent anti-inflammatory effects in preclinical studies. In patients with type 2 diabetes, GKT137831 achieved a significant reduction in hsCRP (an acute phase protein produced in the liver), and tended to reduce plasma levels of serum amyloid protein A and IL-6. Accordingly, changes in hsCRP, fibrinogen, and IL-6 will be evaluated to assess the anti-inflammatory effect of GKT137831.

The role of NOX enzymes in the underlying immunological process is unknown. Nevertheless, it is suggested that NOX1 and NOX4 play a role in some of the immunological pathways underlying PBC. Accordingly, changes in serum IgM, IL-4, IL-12, IL-17A, and interferon γ will be assessed.

2.1.4 Summary of Non-Clinical Studies with GKT137831

A broad range of *in vitro* and *in vivo* pharmacology studies have been conducted to support the use of GKT137831 in several indications. Detailed summaries for all studies are given in the current Investigator's brochure (IB). GKT137831 selectively inhibits human isolated NOX4 (IC_{50} 0.147 μM) and NOX1 (IC_{50} 0.214 μM) but is considerably less active at inhibiting isolated human NOX2 (IC_{50} 3.44 μM), NOX3 (IC_{50} 3.44 μM) and NOX5 (IC_{50} 0.457 μM). This selectivity against NOX2 means GKT137831 does not compromise phagocyte function. Moreover, GKT137831 was inactive against the non-NADPH oxidase flavoprotein xanthine oxidase, and inhibited glucose oxidase with an IC_{50} of 5.7μM, indicating selective inhibition of ROS generation via the NADPH oxidase pathway. Meanwhile, GKT137831 reduced DPPH with an IC_{50} of 20μM, suggesting GKT137831 is a weak electron donor, and may therefore possess weak antioxidant activity. Investigation of potential effects against a broad range of receptors and enzymes confirmed that GKT137831 demonstrated no significant off-target activity, except for significant inhibition of human recombinant 15-lipoxygenase-2. However, a review of available literature did not detect a safety concern.

Similarly, a literature review on the effect of gene deletion of NOX isoforms indicated that no basal phenotype has been associated with NOX1 or NOX4 gene deletion in mice. Specifically, there are no reported defects in fertility, embryonic development, life span, animal behavior or breeding. These observations did not identify potential safety concerns related to inhibition of NOX1 and/or NOX4.

Overall, there are no observations from the available literature to indicate safety concerns associated with chronic pharmacological inhibition of NOX1, NOX4 and NOX5.

Safety pharmacology studies demonstrated that there were no effects on general activity, behavior or respiratory parameters at doses up to 1000 mg/kg of GKT137831 when administered orally. Minimal effects on hERG currents were recorded with a reduction of ~20% at 300 µM with GKT137831 or major active metabolite GKT138184. In addition, GKT137831 was found to be a weak inhibitor (IC50 = 78.8 µM) of the cardiac IKs channel, while the metabolite, GKT138184 has no activity on this channel at 100 µM. GKT137831 and GKT138184 have no significant inhibitory activity on the IKr cardiac ion channel at 300 µM and no activity on INa and ICa channels at 100 µM. Overall, review of the electrocardiogram (ECG) data from the safety pharmacology and toxicology studies did not provide sufficient information that GKT137831 could prolong QTc or QTCF in a way that indicated a definite risk to humans.

The non-clinical PK and absorption, distribution, metabolism and excretion (ADME) of GKT137831 have been investigated *in vivo* in the mouse, hamster, rat and dog, and in animal and human *in vitro* preparations. GKT137831 is a rapidly and extensively orally absorbed compound. In rats and dogs it has moderately low clearance and a volume of distribution approximately similar to that of extracellular fluid and elimination predominates in feces rather than urine.

Significant amounts of the major phase 1 active metabolite GKT138184 have been quantified in animal plasma after oral dosing. The phase 1 metabolism of GKT137831 is likely to be mediated mainly by CYP3A4, and GKT137831 showed the potential to inhibit and induce CYP3A4 *in vitro*. A human drug interaction study indicated that GKT137831 is a weak inhibitor of CYP3A4 although the modest increase in the exposure of midazolam and its metabolites upon repeat dosing of GKT137831 may be due to a combination of mechanisms. Initial *in vitro* studies indicated that GKT137831 was a potent inhibitor of Bile Salt Export Pump (BSEP). Because BSEP inhibitors have the potential to cause cholestatic liver injury, the effects of GKT137831 on bile acid levels were investigated in the clinical drug interaction study. GKT137831 administered for 10 days at 300 mg BID had no effect on bile acid concentration. Furthermore, GKT137831 did not cause hepatocellular injury in toxicology studies. Because of these discrepant *in vitro* and *in vivo* results, the *in vitro* BSEP assay was repeated and did not confirm the initial data. It is therefore likely that GKT137831 is not a potent inhibitor of BSEP. Other *in vitro* transporter studies indicate that there is no requirement for additional testing prior to further phase 2 studies.

GKT137831 has undergone a comprehensive toxicology testing programme which has demonstrated that there is no genotoxicity liability with either the parent or the active metabolites. In chronic studies in both rat and dog the potential toxicity of GKT137831 following up to 6 months of daily oral dosing was explored. The no observed adverse effect level (NOAEL) in rats was established at 1000 mg/kg/day, the highest dose tested and findings included minimal or slight decreases in red blood cells and slight increases in platelets, modest increases in total plasma bilirubin and a small increase above control in the incidence of foamy macrophages in the lung which was of minimal severity grading. No such difference between treated and control animals was

observed in animals subjected to a 5-week treatment free period after 6 months of dosing and these effects were thus not considered of toxicological significance.

The dog was the most sensitive species and the NOAEL was established at 150 mg/kg/day in the 26 week study. Treatment related effects in the dog studies were confined to ECG changes, effects on thyroid hormones, and effects on hematology parameters/bone marrow. Changes in liver function tests observed were generally considered to be of minimal severity and reversible and led to no liver pathologies and are consequently not considered to be of toxicological relevance. In the 28 Day study, QTc(F) prolongation was observed in high dose dogs (1000 mg/kg) and there was a single animal with an AV-block. The QTc prolongation was observed at both measurement times (Day 1 and Day 23) but the increase at the Day 23 reading was limited and seemed to affect only 2/6 treated high dose animals. Although there were no such ECG alterations observed in the 13 Week study, measurements in high dose dogs taken at Week 1 and 4 of the 26 Week study (animals still on 500 mg/kg/day) showed QTc (VdeW) increases of up to 20 msec. There were no QTc prolongation observed after the dose reduction to 300 mg/kg/day at week 13 and 26. The absence of any clear treatment-related ECG alterations at both the NOAEL of 150 mg/kg/day and the HTD (Highest Tolerated Dose) of 300 mg/kg/day in the 26 Week dog study and in already conducted clinical trials suggest that any such liability will not compromise subject safety in early stage clinical studies. Effects on bone marrow were seen at 500 mg/kg/day, the highest dose tested in the 26 week study and in one animal resulted in a severe non regenerative anemia which lead to euthanasia. Effects were restricted to the erythroid lineage and were preceded by a marked reduction in reticulocytes indicating the potential to monitor any potential effect in the clinic.

Reproductive toxicology studies have been performed in rats and rabbits. No treatment-related embryo-fetal findings in any dose group were seen in rats at doses of up to 1000 mg/kg/day. The NOAEL for maternal toxicity was established at 1000 mg/kg/day and for embryo-fetal toxicity was 300 mg/kg/day. In rabbits, GKT137831-related maternal mortality and severe effects on food consumption and body weight were seen at 1000 mg/kg/day, but there was no evidence of embryo-fetal toxicity at any dose level. The NOAEL for maternal and embryo-fetal toxicity was considered to be 300 mg/kg/day.

Overall, this body of non-clinical data suggests that NOX1/4 inhibition with GKT137831 may represent an attractive therapeutic strategy for a broad range of fibrotic disorders.

2.1.5 Summary of Clinical Studies with GKT137831

2.1.5.1 Healthy Volunteers Program

A total of four phase 1 studies have been completed in healthy male subjects. The program was designed to examine the safety and pharmacokinetics of single and multiple ascending doses of GKT137831, the potential for drug interactions using the CYP3A4 probe substrate midazolam, as well as the relative bioavailability of micronized and unmicronized GKT137831 and the potential interactions with food.

In the phase 1 program, a total of 105 male subjects were exposed to GKT137831, and ranged with age from 18 to 49 years. Doses of GKT137831 ranged between 10 mg and 1800 mg and for up 10 successive days in the repeating dose study. The overall safety results indicate good systemic and local tolerability of single or repeated doses of GKT137831.

Overall subjects exposed to GKT137831 had a low incidence of AEs. Most reported AEs were mild in intensity, self-limiting and considered to be unrelated to GKT137831. The most frequently reported AEs were occurrences of nonspecific headache related events. Other reported AEs were mainly mild common infections unrelated to GKT137831. There were no deaths or serious adverse events (SAEs) reported during the course of these studies. There were no changes in vital signs, ECG, hematology, clinical chemistry or urinalysis associated with GKT137831. Thyroid hormones were specifically investigated in a multiple ascending dose study. All mean values remained within the normal range, and changes compared to baseline showed no trend over time suggestive of a relationship with GKT137831.

The pharmacokinetics of GKT137831 were consistent in all phase 1 studies. Exposure to GKT137831, measured by mean AUC and C_{max}, whether after single or repeat administration, increased in a broadly dose proportional manner or less than dose proportional manner. No increased exposure was seen upon single administration of doses higher than 900 mg. After a single dose of 300 mg in either the fasted state or with a non-high fat meal, the mean AUC_{0-t} ranged from 24,100 to 38,000 ng.h/mL. Administration of micronized or unmicronized drug substance did not appear to have any notable impact on plasma exposure. Administration with food appeared to reduce inter-subject variability in exposure, and administration with a high-fat meal increased the mean AUC of GKT137831 by approximately 25%. No reduction in mean exposure was seen upon repeat dosing either at daily doses of 100-900 mg or twice-daily doses of 300-400 mg. A slight accumulation in AUC_{0-t} was measured between the first and last doses upon twice daily dosing, consistent with the increased dosing frequency.

2.1.5.2 Phase 2 Program

A multicenter double blind randomized phase 2 study has been conducted to evaluate the efficacy and safety of GKT137831 in the treatment of DKD and persistent albuminuria. A total 136 subjects were randomized in the study. In the GKT137831 arm, 68 subjects received 100 mg BID for 6 weeks followed by 200 mg BID for a further 6 weeks, representing over 15.22 subject years in GKT137831 exposure.

The trial did not meet its primary endpoint. At the end of the treatment period, no difference could be detected between GKT137831 and placebo in the changes of albuminuria level from baseline. GKT137831 did not appear to affect other measures of renal function and injury, including serum creatinine, eGFR, and urine MCP-1. Reasons for the lack of efficacy on the primary endpoint may include the short treatment period and/or under-dosing during the first half of the trial (see Section 2.2).

Nevertheless, the trial met several predefined secondary endpoints, indicating that GKT137831 is pharmacodynamically active in humans. Specifically, subjects receiving GKT137831 had a statistically significant reduction in GGT and hsCRP levels, two markers which predict mortality in patients with DKD. Furthermore, subjects receiving GKT137831 also showed a robust trend for lower levels of serum amyloid protein A, plasminogen activator inhibitor type 1, and triglyceride levels, compared to subjects receiving placebo. These changes are potentially related to a systemic anti-inflammatory effect, and/or reduced severity of non-alcoholic fatty liver disease in these subjects with type 2 diabetes and excess body weight. Trends toward reduced neuropathic pain and erectile dysfunction were observed but did not reach statistical significance.

In this phase 2 study, GKT137831 was well tolerated. The reporting rate of AEs was low with fewer than 50% of subjects reporting AEs during the course of the study. In GKT137831-treated subjects, most Treatment-Emergent Adverse Events (TEAEs) were self-limiting, mild in severity, not treatment-related and resolved rapidly. The most commonly reported AEs were events related to respiratory tract infections. Other reported events were single occurrences experienced by 1 or 2 GKT137831 subjects. There was no evidence of dose-related increase in the occurrence of TEAEs. The only notable safety finding was a statistically significant, albeit slight and non-clinically significant (~2.5 mm Hg) increase in diastolic blood pressure (DBP). A trend for a marginal (~3 mm Hg) increase in systolic blood pressure (SBP) was also observed, but did not reach statistical significance. Finally safety signals related to findings made in previous toxicology studies were not confirmed. In particular, there were no safety signals related to thyroid function, liver function, bone marrow function, or cardiac conduction. No bone marrow toxicity, cardiac toxicity, liver toxicity, or renal toxicity was observed.

Upon review of all available safety data, the evaluation of GKT137831 at doses up to 200 mg BID in 68 subjects with DKD indicated a good tolerability and safety profile of GKT137831 compared with placebo.

2.2 Rationale

The proposed doses of 400 mg OD and 400 mg BID were selected to achieve target plasma exposure levels in a majority of study subjects. The plasma exposure target was defined according to the exposure achieved in mice by the maximally effective dose. There is considerable experience with GKT137831 in animal models of inflammatory and fibrotic disorders. Over 35 peer-reviewed manuscripts have been published to date, with an average impact factor >9. In these studies, and as presented in the IB, dose of 20 to 30 mg/kg achieved maximal efficacy. This was particularly well demonstrated in the OVE26 and Akita models of DKD, or in a model of acute CCL4-induced liver injury. However, in some instances, trends for higher efficacy were observed at higher doses. For instance, 60 mg/kg tended to be more effective than 5 and 20 mg/kg in the STAM models of non-alcoholic steatohepatitis, and in the MDR2$^{-/-}$ model of cholestatic liver injury and fibrosis. In the bile duct ligation model, 60 mg/kg was as effective as 120 mg/kg. Altogether, this data indicates that the maximally effective doses range from 20 to 60 mg/kg in murine models.

Table 1 highlights the modelled steady state exposure levels for the proposed clinical doses. These exposure estimates were based on a population PK model developed based on the PK data obtained in the completed phase 2 trial, and compared against single dose exposure levels in mice.

Table 1 Predicted Typical Parent and Metabolite Exposures based on Parent and Metabolite Mouse and Human Population PK Models

	Species			
	Mouse		Human	
Dose	20 mg/kg	60 mg/kg	400 mg OD	400 mg BID
AUC_{24} (ng.hr/mL)	25,766	77,299	53,091* 28,423-98,231	103,967* 56,589-187,098

* Median (10th-90th)

These exposure estimates indicate that more than 90% of subjects allocated to the 400 mg OD dose are predicted to have plasma exposure levels above the average exposure achieved in mice by the 20 mg/kg dose (i.e. the less conservative target exposure level). A majority of subjects allocated to the 400 mg BID dose are predicted to have plasma exposure levels above the average exposure achieved in mice by the 60 mg/kg dose (i.e. the more conservative target exposure levels). In addition, the 2 proposed doses should achieve plasma concentrations above $5xIC_{50}$ (200 ng/mL) for the full dosing interval. As shown in the rat quantitative whole-body autoradiography study, high levels of radiolabeled drug-related material were observed in liver tissue where it achieves concentrations that are 3-5 times higher than in other organs, such as kidney or lung. This tissue distribution pattern and elimination route matches the target organ in PBC patients. Based on recently reported trials, which applied very similar eligibility criteria, the subjects enrolled in this trial will mainly have mild PBC. Nevertheless, the impact of potential hepatic drug accumulation will be assessed by careful monitoring of liver function tests and drug concentration in plasma.

The proposed dosing regimens are supported by the available non-clinical and clinical data. Doses up to 1800 mg were tested in the single ascending dose phase 1 study. Subsequently, doses of up to 900 mg/day were tested in the multiple ascending dose study. In these studies conducted in healthy subjects, GKT137831 was well tolerated. No dose limiting toxicities and no safety signals were detected.

In the completed phase 2 trial in DKD patients, 100 mg BID was evaluated over the first 6 weeks, and 200 mg BID was evaluated over the following 6 weeks. The safety profile of GKT13783 was again very favorable. Subjects receiving GKT137831 reported a lower incidence of AEs compared to subjects receiving placebo. This reduction (in the range of 40% for TEAEs) was especially marked for moderate to severe TEAEs. In addition, a low number of serious adverse events were reported in subjects receiving GKT137831 (3 SAEs) or placebo (7 SAEs). No safety signals were identified in

subjects receiving GKT137831, except for a marginal yet statistically significant increase in DBP. GKT137831 has not been shown to increase blood pressure in toxicology studies or in healthy subjects. Therefore, these findings may be due to chance, or specific to patients with DKD. Meanwhile, the toxicology signals identified in rat and dog toxicology studies were not observed in human. In particular, GKT137831 did not cause consistent changes in ECG parameters, liver function tests, thyroid function tests, and hematology parameters. Overall, these results indicated that GKT137831 is very well tolerated when administered orally for up to 12 weeks at doses up to 400 mg/day. In our opinion, the combined safety data obtained in human subjects support the evaluation of GKT137831 at higher doses and for longer treatment durations.

Specifically, the combined non-clinical and clinical data support the two doses proposed for this trial. The 400 mg OD dose corresponds to the daily dose evaluated during the second 6-week period in the completed phase 2 study. As indicated above, this dosing regimen was very well tolerated in the study population, which consisted of type 2 diabetics presenting with multiple diabetic complications and concomitant medications. Considering the very good safety profile of GKT137831 in this susceptible population, the 400 mg BID was selected to explore a higher dose range while maintaining adequate safety margins based on animal data. Specifically, the dose of 400 mg BID is estimated to provide a safety margin of 2.1 and 3.2 for AUC and C_{max}, respectively (see Table 2).

Table 2 Safety Margins of Predicted Human Exposure Compared with Exposures at the NOAEL in Dog Safety Study GSN000157

PK parameter [GKT137831 + GKT138184]	Exposure at toxicology NOAEL (26 week dog)[a]	Predicted human exposure at 400 mg b.i.d[b]. [safety margin]	Predicted human exposure at 400 mg q.d[b] [safety margin]
AUC (µg.h/mL)	220	104 [2.1]	53 [4.1]
C_{max} (µg/mL)	44	14 [3.1]	14 [3.1]

a: combined [GKT137831 + GKT138184] value in non-acidified plasma at Week 26 in study GSN000157 at NOAEL dose (150 mg/kg/day).
b: predicted median [GKT137831 + GKT138184] exposures based on 1000 simulations of GKT137831 and GKT138184 in human population PK models.

We anticipate a low enrollment rate for this trial, with a total enrollment period of around 7-8 months. Considering the number of treatment arms, the exposure of subjects to the top dose will accrue at a slow pace (an average of approximately 3-5 subjects will be randomized to the 400 mg BID dose every month). We expect even slower incremental exposure during the early stages of the trial conduct due to the progressive activation of investigational centers. Careful monitoring of study subjects, as well as periodical review of safety data by the trial Safety Monitoring Board will ensure the safety of participating subjects. Of note, study subjects will be monitored more frequently in the early phase of the trial, with visit intervals of 2 weeks, 4 weeks, and subsequently 6 weeks.

2.3 Potential Risks and Benefits

As summarized above, GKT137831 has been extensively investigated in pre-clinical studies as well as in four studies in healthy volunteers and one study in subjects with Type 2 diabetes. GKT137831

was well tolerated when administered for up to 12 weeks at a dose of up to 200 mg BID. No safety signal or dose-limiting toxicities were identified in these studies. In particular, safety signals identified in toxicology studies were not detected. Specifically, there was no detectable drug effect on thyroid function, bone marrow function, liver function tests, or cardiac conduction. The careful extension of the treatment duration (i.e. 24 weeks) and dose increase to 400 mg BID are supported by the favorable safety data obtained in previous clinical studies. As a general precaution, however, appropriate safety endpoints have been built into this study for frequent monitoring (see Section 3). In addition, a SMB will carefully review the emerging study data on a regular basis in order to detect any safety signals early and take appropriate action according to the SMB charter.

Nevertheless, specific risks related to the subject population exist. GKT137831 caused minor elevations of total bilirubin in dog toxicology studies. These changes were transient and reversible, and were not associated with biochemical or pathological evidence of hepatocellular injury. The underlying cause for these changes has not been identified, and may involve competition for, or inhibition of, UGT enzymes which are responsible for the glucuronide conjugation. In phase 1 and phase 2 trials, GKT137831 did not cause elevations in total bilirubin. Nevertheless, this study will evaluate a higher dose over a longer treatment period, and drug accumulation in liver tissue is anticipated in PBC patients. Accordingly, the incidence of drug induced liver injury (DILI) will be carefully monitored as outlined in Section 6.3.1.

In addition, a case of non-regenerative anemia was observed in the 26 week dog toxicology study. Accordingly, hematological parameters were carefully monitored in subsequent phase 1 and phase 2 studies. No safety signal related hematological parameters were detected in these trials. Nevertheless, hematological parameters will be carefully monitored as described in Section 6.3.2.

While NOX1/4 inhibition represents an attractive therapeutic strategy in patients with PBC, actual therapeutic benefits in PBC patients remain to be demonstrated.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVE	ENDPOINT (OUTCOME MEASURE)
To evaluate the efficacy of oral GKT137831 in comparison with placebo, in subjects with PBC receiving UDCA and with persistently elevated serum ALP.	Primary efficacy endpoint: • The percent change from baseline to Week 24 (Visit 7) in serum GGT. Secondary efficacy endpoints: • Absolute and percent change in serum GGT from baseline to each assessment. • Absolute change in ELF score from baseline to Weeks 12 and 24. • Absolute and percent change in serum ALP from baseline to each assessment. • Absolute and percent change in serum levels of hsCRP, and fibrinogen, from baseline to each assessment. • Absolute and percent change in serum ALT, AST, conjugated and total bilirubin, from baseline to each assessment. • Absolute and percent change in the FIB-4 and APRI scores, from baseline to each assessment (FIB-4: age (years) x AST (IU/L)/(platelet count (10^9/L) x (ALT (IU/L)1⁄2, APRI: AST (IU/L)/ upper normal limit AST)x100/platelet count (10^9/L). • Absolute and percent change in liver stiffness as assessed by transient elastography (FibroScan® or similar technology), from baseline to Week 24, in subjects with values at baseline and Week 24. • Absolute and percent change in serum levels of collagen fragments indicative of collagen formation and degradation, from baseline to Weeks 12 and 24. • Absolute and percent change in total bile acids from baseline to Week 12 and 24.

	Tertiary efficacy endpoints: • Absolute and percent change in Quality of Life (QoL) and Pruritus scores based on the PBC-40 and Pruritus Visual Analogue Scale (VAS), from baseline to Weeks 12 and 24. • Proportion of subjects achieving a 15, 20, 30 and 40% reduction in serum ALP from baseline to each assessment. • Proportion of subjects who meet the definition of PBC responder criteria applying the Paris I, Toronto I, Toronto II, Toronto III, Toronto IV, Mayo II, and Barcelona disease prognostic risk criteria at Weeks 12 and 24. Exploratory Endpoints: • Optionally, absolute and percent change in serum C4 and FGF19 from baseline to Weeks 12 and 24. • Optionally, absolute and percent change in serum interleukin (IL)-6 and cytokeratin-18 (CK-18), from baseline to Weeks 12 and 24. • Optionally, absolute and percent change in serum IgM, IL-4, IL-12, IL-17A, and interferon γ, from baseline to Weeks 12 and 24. • Optionally, assessment of metabolomics signatures. • Optionally, assessment of additional biomarkers of interest.
SECONDARY OBJECTIVES: To evaluate the safety of oral GKT137831 in comparison with placebo, in subjects with PBC.	• Subjects with AEs from starting IMP to 28 days after the last administration. • Clinical laboratory evaluations at Screening, baseline/Day 1 and Weeks 2, 6, 12, 18, 24 and 28. • Urinalysis at Screening and Weeks 12, 24 and 28. • Thyroid stimulating hormone (TSH) measured at baseline/Day 1 and Weeks 12 and 24. • Pulse rate, SBP and DBP at Screening, baseline/Day 1 and Weeks 2, 6, 12, 18, 24 and 28. • Body weight at baseline/Day 1 and at Week 24. • 12-lead ECG during Screening and at Weeks 2, 12, and 24.
To estimate the population pharmacokinetics (PK) of GKT137831 and explore any	• Plasma concentrations of GKT137831 and its main phase 1 metabolite, GKT138184. The plasma concentrations will be subjected to population PK analysis to estimate population PK parameters such as clearance

potential PK-PD relationships in this subject population.	and volume of distribution and associated inter-individual variability (IIV), and to determine predictors of IIV. PK-PD analysis will be carried out using the primary endpoint and selected secondary endpoints in order to explore any potential PK-PD relationships. Exploratory Endpoint: • Additional biomarkers of interest will be measured in plasma or serum. The identity of these exploratory markers will be defined in response to findings described in the literature or obtained in this study. These markers will not have diagnostic or prognostic value and will not have an established normal range according to the World Health Organization (WHO).
To explore any relationship between genetic parameters and therapeutic responses in a subset of subjects.	• Genetic and pharmacogenetic research may be conducted on the DNA samples collected at baseline/Day 1 from subjects who sign an optional, additional informed consent. In particular, the impact of polymorphisms in the NOX1 and/or NOX4 genes on pharmacodynamic and therapeutic responses may be assessed.

4 STUDY DESIGN

This will be a double-blind, randomized, placebo-controlled, multicenter, parallel group phase 2 trial assessing a 24-week period of treatment with oral GKT137831 administered in addition to standard of care medication (UDCA) in subjects with PBC.

Subjects will be assessed for their eligibility during the screening period (Visit 1), which will last up to 4 weeks, until the baseline/Day 1 visit (Visit 2).

Eligible subjects will be randomized to oral GKT137831 (400 mg OD or 400 mg BID) or placebo, according to a 1:1:1 randomization ratio, stratified at study entry by disease severity defined as baseline serum GGT < 2.5 x the ULN or ≥ 2.5 x ULN.

Subjects will self-administer orally 400 mg OD or 400 mg BID of GKT137831 or matching placebo for a total of 24 weeks.

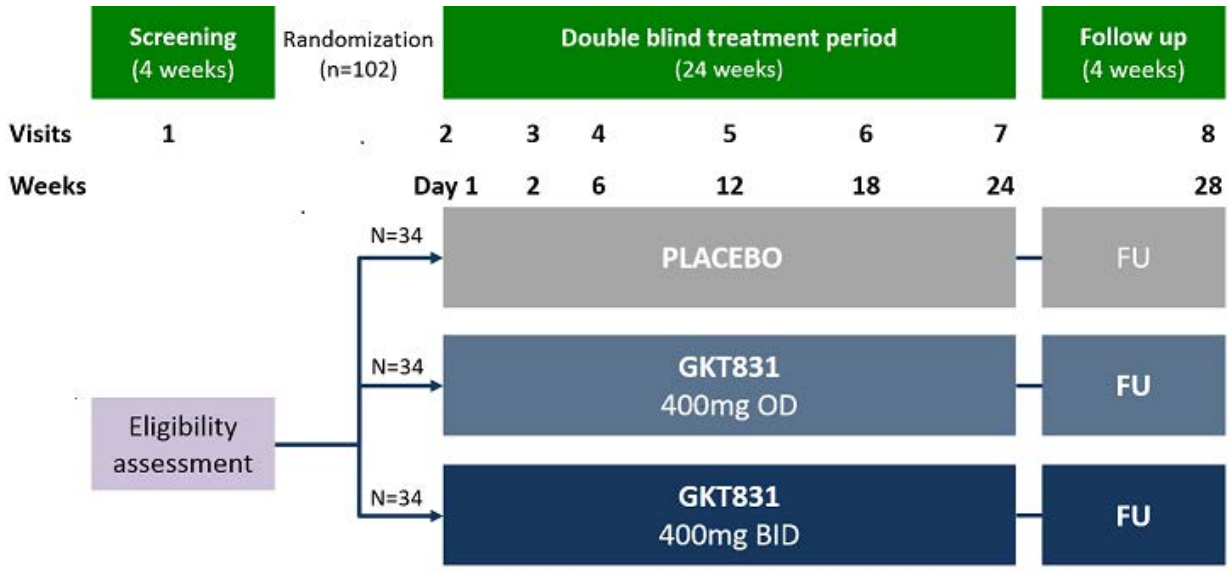
Baseline assessments will be performed at baseline/Day 1 (Visit 2). The 24-week treatment period will include assessments after 2 weeks of treatment (Visit 3), after 6 weeks of treatment (Visit 4), after 12 weeks of treatment (Visit 5), after 18 weeks of treatment (Visit 6) and after 24 weeks of treatment (End of Treatment/Visit 7). Subjects will be followed up for 28 days after the end of treatment (Week 28/Visit 8), totaling 6 post-baseline visits. Subjects who discontinue treatment before Week 24 will have an Early Termination visit (premature end of treatment).

Pharmacokinetic samples will be taken at baseline, Week 2, Week 12 and Week 18 (see Section 8.2.2 for the sampling time points).

Subjects will be taking a stable dose of UDCA at enrollment and will continue their UDCA treatment at a stable dose (no changes at all) during the treatment period.

A Safety Monitoring Board will oversee the conduct of the study to ensure the safety of participating subjects (see Section 9.7 for more details). An interim analysis will be conducted when approximately 90 subjects have completed their Week 6 visit (see Section 10.3).

Figure 1: Study Design Flowchart



5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Population

Subjects with primary biliary cholangitis receiving a stable dose of UDCA and with persistently elevated ALP, who meet all of the inclusion criteria listed in Section 5.2 and none of the exclusion criteria listed in Section 5.3, may be included in this study. A total of 102 subjects will be randomized.

5.2 Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Male or female aged 18 to 80 years, inclusive.

2. Willing and able to give written informed consent and to comply with the requirements of the study.

3. PBC diagnosis as demonstrated by the presence of ≥ 2 of the following 3 diagnostic factors:

    a) History of elevated ALP levels (>ULN) for at least 6 months

    b) Positive AMA titer or if AMA negative or in low titer (<1:80) PBC specific antibodies (anti-GP210 and/or anti-SP100 and/or antibodies against the major M2 components [PDC-E2, 2-oxo-glutaric acid dehydrogenase complex])

    c) Liver biopsy consistent with PBC (based on historic liver biopsy), including non-suppurative, destructive cholangitis affecting mainly the interlobular and septal bile ducts.

4. Serum ALP ≥1.5 x ULN.

5. Serum GGT ≥1.5 x ULN.

6. UDCA treatment for at least 6 months and stable dose for at least 3 months prior to Visit 1.

7. Subjects being treated for pruritus with colestyramine must be on a stable dose of colestyramine for at least 8 weeks prior to baseline/Day 1 (Visit 2). Subjects must be willing and able to take colestyramine at least 2 hours before or after study medication.

8. Female subjects of childbearing potential must use a highly effective method of contraception to prevent pregnancy for 4 weeks before randomization and must agree to continue strict contraception for 90 days after last administration of IMP (see Section 5.4). Male participants with female partners of childbearing potential must be willing to use a condom and require their partner to use an additional form of adequate contraception as approved by the Investigator.

This requirement begins at the time of informed consent and ends 90 days after the last administration of IMP. Male study participants must also not donate sperm from baseline until 90 days after the last administration of IMP.

5.3 Exclusion Criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. A positive pregnancy test or breast-feeding for female subjects.
2. Any hepatic decompensation, defined as a past or current history of hepatic encephalopathy, gastrointestinal tract bleeding due to esophageal varices, or ascites.
3. INR > 1.2 unless subject is on anticoagulant therapy.
4. ALT > 3 x ULN.
5. Total bilirubin > 1 x ULN.
6. Planned or current plasmapheresis or other extra-corporeal treatments (e.g., MARS) for treatment-refractory pruritus.
7. History of liver transplantation, current placement on a liver transplant list or current MELD score ≥ 15.
8. Cirrhosis with complications, including history or presence of: spontaneous bacterial peritonitis, hepatocellular carcinoma.
9. Hepatorenal syndrome (type I or II) or Screening serum creatinine > ULN.
10. Competing etiology for liver disease (e.g., hepatitis C, active hepatitis B, NASH, ALD, autoimmune hepatitis, primary sclerosing cholangitis, Gilbert's Syndrome).
11. Subjects receiving prohibited medications within 3 months of Screening (Visit 1) according to the list (a, b and c) provided in Section 6.6.2.
12. Treatment with any investigational agent within 4 weeks of Screening (Visit 1) or 5 half-lives of the IMP (whichever is longer).
13. A history of long QT syndrome.
14. Evidence of any of the following cardiac conduction abnormalities during the screening period:
    - A QTc Fredericia interval >450 milliseconds for males and >470 milliseconds for females.
    - A second or third degree atrioventricular block not successfully treated with a pacemaker.
15. History of cancer in the preceding 5 years, except adequately treated non-melanoma skin cancer, carcinoma *in situ* of the cervix, *in situ* prostate cancer, *in situ* breast ductal carcinoma, or superficial bladder cancer stage 0).

16. The occurrence of any acute infection requiring systemic antibiotic therapy within 2 weeks prior to Screening (Visit 1), or HIV infection.
17. A history of bone marrow disorder including aplastic anemia, or marked anemia defined as hemoglobin < 10.0 g/dL (or 6.2 mmol/L).
18. Any condition which, in the opinion of the Investigator, constitutes a risk or contraindication for the participation of the subject in the study, or which could interfere with the study objectives, conduct, or evaluation.

5.4 Contraception

Female subjects of childbearing potential must use a highly effective method of contraception to prevent pregnancy for 4 weeks before randomization and must agree to continue strict contraception for 90 days after the last administration of IMP.

For the purposes of this trial, women of child bearing potential (WOCBP) are defined as “All female subjects after puberty unless they are post-menopausal (defined as amenorrhea for 12 months with documented date of last monthly period) or are surgically sterile (i.e., bilateral tubal occlusion).” For female subjects aged <55 who are considered post-menopausal and who are not on concomitant estrogen replacement therapy, confirmation of postmenopausal status will be required with follicle stimulating hormone (FSH) test results in the postmenopausal range for age at Screening (Visit 1).

Highly effective contraception is defined as methods which can achieve a failure rate of less than 1% per year when used consistently and correctly. Such methods include: combined (estrogen + progestogen) hormonal contraception (oral/intravaginal/transdermal); progestogen-only hormonal contraception (oral/injectable/implantable); intrauterine device (IUD); intrauterine hormone-releasing system (IUS); vasectomized partner (provided that partner is the sole sexual partner of the WOCBP study subject and that the vasectomized partner has received medical assessment of the surgical success); or sexual abstinence (defined as refraining from heterosexual intercourse from 4 weeks before randomization until 90 days after the last administration of IMP and only if this is the preferred and usual lifestyle of the subject).

WOCBP must have a negative serum pregnancy test at Screening (Visit 1) and a negative urine pregnancy test at baseline/Day 1 (Visit 2) before dosing.

Male participants with female partners of childbearing potential must be willing to use a condom and require their partner to use an additional form of adequate contraception as approved by the Investigator, such as an established form of hormonal contraceptive, a diaphragm or cervical/vault cap, IUD, or sponge with spermicide. This requirement begins at the time of informed consent and ends 90 days after last administration of IMP. Male study participants must also not donate sperm from baseline until 90 days after last administration of IMP.

5.5 Strategies for Recruitment, Retention and to Improve Adherence to Protocol

Sites will be selected based on their access to the PBC population. Several strategies may be implemented as enhancements to support subject identification and recruitment, including advertising through patient advocacy groups/networks, on Orphanet and other health-related social networks and early engagement with Key Opinion Leaders in each country to provide PBC expertise as well as physicians actively treating patients in the selected countries to encourage potential referrals.

Genkyotex's designated Clinical Research Organization (CRO), Cmed, will provide ongoing management of enrollment by regular review of site performance. Upon indication a site may benefit from further support, the site and the Clinical Research Associate (CRA) will partner to re-examine the current recruitment strategy.

5.6 Treatment Assignment Procedures

5.6.1 Randomization Procedures

This will be a double-blind, randomized, placebo-controlled, multicenter, parallel group phase 2 trial. A total of 102 subjects will be randomized and allocated to placebo or one of the 2 active treatment arms, according to a 1:1:1 randomization ratio, stratified at study entry by disease severity defined as baseline serum GGT < 2.5 x ULN or ≥ 2.5 x ULN). Accordingly, approximately 34 subjects will be allocated to each of the 3 treatment arms.

A master reproducible randomization list will be produced by or under the responsibility of Cmed accounting for block size and stratification. The IWRS will assign a unique randomization number in ascending, sequential order (with associated treatment arm) to the subject, based on the pre-determined randomization schedule. The system will assign the pre-determined blocks of randomization numbers for each stratification level to ensure similarly balanced treatment groups. Assignment will be in sequential order within blocks and stratification level. The investigator will enter the randomization number in the electronic case report form (eCRF).

5.6.2 Blinding Procedures

This is a double-blind study: the Sponsor, subjects, investigator staff, persons performing the assessments and data reviewers and statisticians will remain blinded to the identity of the study treatments.

The identity of the study treatments will be concealed by the use of IMPs which are all identical in packaging, labeling, schedule of administration, appearance and odor.

Randomization data will be kept strictly confidential, and will be accessible only to authorized personnel (e.g., unblinded pharmacist or authorized designee), until unblinding of the trial as described in the statistical analysis plan (SAP).

Unblinding will only occur for the following reasons:

- Subject emergencies (see Section 5.6.3)
- Scheduled and unscheduled safety reviews by the SMB (see Section 9.7)
- At the time of the interim analysis (see Section 10.3)
- At the conclusion of the study.

5.6.3 Emergency Breaking of Assigned Treatment Code

If an emergency unblinding becomes necessary, the Investigator should notify the Sponsor/Medical Monitor prior to unblinding, if possible, unless identification of the IMP is required for emergency therapeutic measures. The result of the code break should not be revealed to the Sponsor/Medical Monitor. Unblinding will be performed through the IWRS. The Investigator will access the IWRS to access the subject's treatment code. The Investigator may always, for urgent safety reasons, break the blind at any time during the conduct of the study. If the blind is broken, the date, time and reason must be recorded in the subject's eCRF and any associated AE report.

The unblinded treatment code must not be recorded in the eCRF.

5.6.4 Reasons for Withdrawal

Subjects will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons. Any withdrawal must be fully documented in the eCRF, and should be followed up by the Investigator.

The Investigator may withdraw a subject at any time if this is considered to be in the subject's best interest.

A subject MUST be withdrawn for the following reasons:

- Pregnancy or breast feeding.
- A change in UDCA dose during the treatment period.
- Confirmed or suspected DILI, as defined in Section 6.3.1.
- Confirmed or suspected liver decompensation, defined as hepatic encephalopathy, gastrointestinal tract bleeding due to esophageal varices, or ascites.
- Confirmed Grade ≥ 2 severity anemia without documented alternative cause, as defined in Section 6.3.2.
- Grade 3 severity AE recurring or not resolving after a pre-defined interruption and resumption of IMP (see Section 6.3).
- Any Grade 4 severity AE.

- Subject's withdrawal of consent.

The subject MAY be also discontinued for the following reasons:

- Protocol violations, including non-compliance and loss to follow-up.
- Safety reasons.
- Administrative reasons.
- Other: the subject was withdrawn for a reason other than those listed above, such as termination of the study by the Sponsor (see Section 5.6.6).

A subject will be considered to have completed the study when he/she completes the final assessment visit of the follow-up period, i.e., Week 28 (Visit 8).

5.6.5 Handling of Withdrawals

If the IMP is prematurely discontinued (i.e., before Week 24), the primary reason for discontinuation must be recorded in the appropriate section of the eCRF and all efforts must be made to complete and report the observations as thoroughly as possible. A premature end of treatment evaluation should be performed following the subject's withdrawal as described in Section 7.5, and recorded in the early termination eCRF page. A final follow-up visit should be performed within 4 weeks following the early termination visit, as described in Section 7.4.

If a subject fails to return for follow-up, attempts should be made to contact the subject to ensure the reason for not returning is not an AE. Likewise, if a subject decides to discontinue from the study, an attempt should be made to establish that the true reason is not an AE (bearing in mind subjects are not obliged to state their reasons). For subjects considered lost to follow-up, the eCRF must be completed up to the last visit performed.

Ongoing AEs should be followed up in accordance with the procedures described in Section 9.2.4.

Subjects who discontinue early will not be replaced.

5.6.6 Termination of Study

This study may be terminated at any time by the Sponsor because of safety concerns, ethical issues; plans to modify, suspend or discontinue the development of the IMP or serious and/or continued non-compliance with the protocol.

If the study is suspended or terminated, the Sponsor will promptly inform the Investigator, and will also inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. The relevant Institutional Review Board/Independent Ethics Committee (IRB/IEC) will also be informed promptly and provided the reason(s) for the termination or suspension by the Sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

The Sponsor may at any time, at its sole discretion, discontinue a study center for various reasons, including, without limitation, the following:

- Failure of the Investigator to enroll subjects into the study at a reasonable rate.
- Failure of the Investigator to comply with applicable laws and/or pertinent regulations.
- Submission of knowingly false information from the research facility to the Sponsor, CRA, or regulatory authorities.
- Insufficient adherence to protocol requirements.

The Sponsor will issue a written notice to the Investigator, which will contain the reasons for taking such action. If the study center is terminated for non-compliance, the Sponsor will notify the appropriate regulatory authorities.

6 TREATMENT OF SUBJECTS

6.1 Identity of Study Treatment(s)

6.1.1 Description and Formulation

The description of IMP provided to each study center pharmacy is shown in Table 3.

Table 3 Description of Investigational Medicinal Products

Drug code	GKT137831	Placebo
Formulation	Micronized API formulated with excipients in capsules	Matching capsules containing only the excipients
Strength	100 mg	-
Route	Oral	Oral

GKT137831 capsules will contain 100 mg GKT137831 powder formulated with the following excipients per capsule: microcrystalline cellulose, Aerosil® (silicon dioxide), magnesium stearate and hard gelatin.

6.1.2 Packaging and Labeling

The IMP will be packed and labeled in accordance with applicable local regulatory requirements and applicable International Conference for Harmonization (ICH) Good Manufacturing Practice (GMP) and ICH Good Clinical Practice (GCP) guidelines, and to protect the blinded nature of this clinical study. The multi-language tear-off label will be adapted to the size of the IMP package and translated into the appropriate languages.

Child resistant 150 mL high-density polyethylene (HDPE) bottles with a tamper evident seal containing 70 capsules of either 100 mg GKT137831 capsules or matching placebo will be provided to the responsible pharmacist at the study center, who will dispense the bottles of IMP in cardboard packs for each subject, at each dispensing visit, in accordance with the Pharmacy Manual which will be developed according to ICH GCP guidelines and applicable local laws.

6.1.3 Storage and Stability

The IMP must be stored in a cool, dry, area at room temperature (between 15°C and 25°C). At the site IMP must be securely locked and stored with restricted access. Temperature should be controlled during shipment and during storage at study centers. The IMP must not be frozen or stored above 27°C.

6.2 Administration of Study Treatment(s)

At each dispensing visit, each subject will be given individual packs containing 2, 4 or 6 (depending on the visit) IMP bottles (70 capsules of 100 mg GKT137831 and/or matching placebo per bottle), in accordance with Table 4 and the Pharmacy Manual. Each subject will be given a sufficient supply of IMP to last until the next study visit, with some overage (up to 3 days).

Table 4 Quantity of IMP Bottles Dispensed at Each Visit

	Visits	**Visit 2**		**Visit 3**		**Visit 4**		**Visit 5**		**Visit 6**		**Visit 7**
	Week	Baseline/ Day 1		2		6		12		18		24 (End of Treatment)
	IMP Bottles	P	A	P	A	P	A	P	A	P	A	No dispensing
Treatment arm	Placebo	2	_	4	_	6	_	6	_	6	_	No dispensing
	400 mg OD	1	1	2	2	3	3	3	3	3	3	No dispensing
	400 mg BID	_	2	_	4	_	6	_	6	_	6	No dispensing

P = Placebo; A = Active (GKT137831)

The IMP will be orally self-administered BID, once in the morning and once in the evening (aiming for a period of at least 10 hours between doses) with meals or up to 30 minutes after eating a meal.

On the day of the visit, study subjects should not self-administer the IMP as usual but instead should bring the IMP bottles to the clinic (used and unused, see Section 6.5) where he/she will be instructed to self-administer the morning dose after the required pre-dose blood samplings have been performed, fasted. The subject must fast overnight from 10 p.m.

The dose of test product will be 400 mg OD or 400 mg BID for 24 weeks.

Each day during the treatment period, subjects will self-administer 4 capsules in the morning and 4 capsules in the evening.

All subjects will be provided with:

- 2 bottles at baseline/Day 1 (Visit 2) and will self-administer:
    - When allocated to GKT137831 400 mg OD: 4 capsules of GKT137831 100 mg from one bottle in the morning and 4 capsules of placebo from the other bottle in the evening.

- When allocated to GKT137831 400 mg BID: 4 capsules of GKT137831 100 mg from one bottle in the morning and 4 capsules of GKT137831 100 mg from the other bottle in the evening.
- When allocated to placebo: 4 capsules of placebo from one bottle in the morning and 4 capsules of placebo from the other bottle in the evening

- 4 bottles at Week 2 (Visit 3) and will self-administer the IMP as described above.
- 6 bottles at Week 6 (Visit 4), Week 12 (Visit 5) and Week 18 (Visit 6) and will self-administer the IMP as described above.

Bottles will be blinded but will contain distinct identification to specify from which bottles the subject should take the capsules.

Table 5 Investigational Medicinal Product Self-Administration

	IMP Bottle			
4 capsules by intake (oral)	Morning placebo	Morning 100 mg	Evening placebo	Evening 100 mg
Placebo	X		X	
400 mg OD		X	X	
400 mg BID		X		X

6.3 Criteria for Study Treatment Modification or Discontinuation

AEs will be graded according to the CTCAE grading system. If an AE is not listed in the CTCAE, the Investigator will use a similar 4-point scale (Grade 1 = mild, 2 = moderate, 3 = severe and 4 = life threatening), as described in 9.2.1.1.

In case of a Grade 1 or 2 severity AE, the subject will be treated as deemed appropriate by the Investigator, and treatment with the IMP should be continued as scheduled.

In case of a Grade 3 severity AE, administration of the IMP must be interrupted until the severity of the event has resolved to ≤ Grade 2 and then resumed at 100% of the initial dose and schedule. Treatment withheld due to a Grade 3 severity AE will not be made up later. If a Grade 3 severity AE does not resolve to ≤ Grade 2 within 24 hours of IMP interruption, or if a Grade 3 severity AE recurs after resumption of IMP, the subject must be permanently discontinued from treatment (see Section 5.6.4).

In case of any Grade 4 severity AE, administration of the IMP must be permanently discontinued.

Withdrawals will be handled as described in Section 5.6.5.

6.3.1 In case of Confirmed or Suspected Drug Induced Liver Injury

In case of any of the following events:

- Grade ≥ 2 severity liver-related AE, or
- Grade ≥ 3 severity ALT or AST (i.e. ≥ 5x ULN), or
- Grade ≥ 2 severity total bilirubin (i.e. ≥ 1.5x ULN), or
- Grade ≥ 2 severity INR (i.e. ≥ 1.5x ULN)

The investigator will instruct the subject to interrupt IMP administration and to return to the study center within 2-3 days to perform a re-test and to undergo additional investigations (i.e. close monitoring as described in Appendix F) to assess for potential DILI. In case of confirmed or suspected DILI as assessed by the investigator, IMP administration must be permanently discontinued, in particular in the following cases:

- Re-test ALT and/or AST values ≥ 3 x baseline and greater than the ULN associated with new onset total bilirubin ≥ 2 x ULN or INR ≥ 1.5.

If subjects live in a remote area, they can be tested locally and the results communicated to the investigator site promptly.

In case of non-confirmed DILI, IMP administration will be resumed in accordance with the protocol and subject will remain under close monitoring as deemed necessary by the investigator.

Any confirmed or suspected DILI event will be closely monitored by the investigator and reported to Sponsor following the same procedure as for SAEs, as described in Section 9.5.

Withdrawals will be handled as described in Section 5.6.5.

6.3.2 In case of Anemia

In case of grade ≥ 2 severity anemia, the Investigator will instruct the subject to interrupt IMP administration and to return to the study center within 7 days to perform a re-test of absolute reticulocyte count.

If the re-test value is below 50% of baseline, and/or if an alternative cause for the anemia cannot be documented, treatment will not be resumed. IMP administration will be resumed only if the re-test value for the absolute reticulocyte count is ≥ 50% of the baseline value and an alternative cause for the anemia can be documented. The subject will continue to be closely monitored until normalization, i.e. return to baseline values.

Withdrawals will be handled as described in Section 5.6.5.

6.3.1 In case of Drug Induced Hypothyroidism

In case of TSH ≥ 10 mIU/L, the Investigator will instruct the subject to interrupt IMP administration and to return to the study center within 2-3 days to perform a re-test of TSH and measure free T4.

If the re-test value is ≥ 10 mIU/L and the subject presents with signs and symptoms consistent with hypothyroidism or if overt biological hypothyroidism is confirmed (i.e. TSH ≥ 10 mIU/L and reduced free T4), treatment will not be resumed. If drug induced hypothyroidism is not confirmed, IMP administration will be resumed as per protocol. The subject will continue to be closely monitored until normalization, i.e. return to baseline values.

Withdrawals will be handled as described in Section 5.6.5.

6.4 Accountability Procedures for the Study Treatment(s)

Records will be maintained of the delivery of IMP to the study centers, the inventory at the study centers, the allocation to, use of, and return by each subject to the study center, and the return to the Sponsor.

These records will include dates, quantities, batch numbers, expiry dates and the unique code numbers assigned to the IMP and to the study subjects. No subject diary is planned.

The Investigator will be responsible for ensuring the records adequately document that the subjects are provided the doses specified in the protocol and all IMP received from the Sponsor is reconciled.

6.5 Subject Compliance

The assigned IMP, dosage, timing and mode of administration may not be changed, except as described in Section 6.3. Any departures from the intended regimen must be recorded in the eCRF.

IMP accountability and subject compliance will be documented throughout the study using study-specific IMP dispensing and return record forms.

Subjects will be asked to return all used IMP, including empty and partially used containers. IMP will be dispensed at baseline/Day 1 (Visit 2), Week 2 (Visit 3), Week 6 (Visit 4), Week 12 (Visit 5) and Week 18 (Visit 6). At Week 2 (Visit 3), Week 6 (Visit 4), Week 12 (Visit 5), Week 18 (Visit 6) and Week 24 (Visit 7), the unused IMP dispensed to the subject at the previous visit will be returned to the Investigator. The Investigator will determine compliance by capsule count per bottle and batch number. Subjects exhibiting non-compliance as assessed by capsule counts will be counseled on the importance of good compliance to the study dosing regimen. Drug non-compliance is defined as taking less than 80% or more than 120% of IMP during the double-blind treatment period.

6.6 Concomitant Medications/Treatments

6.6.1 Required Concomitant Medications

Subjects must be taking UDCA for at least 6 months and be on a stable dose (defined as no changes) for at least 3 months prior to the first Screening visit (Visit 1). All efforts should be made to keep the dose of UDCA stable during the treatment period. Changes in UDCA dose during the treatment period will lead to the early discontinuation of the study subject.

6.6.2 Prohibited Medications

Note: these are not exhaustive lists. The FDA and other agencies maintain current lists, which can be referred to via their websites. Furthermore, these lists will evolve as new drugs come to market and more is learned about the pharmacology of GKT137831 and other medications. Therefore, they should be regarded as a minimum set of excluded and pre-cautioned concomitant medications.

The following medications are prohibited within 3 months of Screening (Visit 1) and during the double-blind treatment period:

a) OCA, budesonide and other systemic corticosteroids, colchicine, mycophenolate mofetil, azathioprine, sulfasalazine, leflunomide, cyclophosphamide, fenofibrates and other fibrates, valproate, isoniazid, and nitrofurantoin.

b) Any biologic agent within 12 weeks or 5 half-lives prior to Screening (Visit 1), whichever is longer. In the case of rituximab, use within 168 days (24 weeks) of Screening or no recovery (level < 20% of pre-rituximab levels or below lower level of normal, whichever is lower) of CD19-positive B lymphocytes if the last dose of rituximab has been more than 24 weeks prior to Screening.

c) Patients taking the following medications which are OAT1 and OAT3 substrates are not allowed on the study because of the underlying conditions these medications are used to treat: methotrexate, probenecid, aminohippurate, cephradine, cidofovir, adefovir, oseltamivir, acyclovir, ganciclovir, benzylpenicillin, cefaclor, ceftizoxime, bumetanide, famotidine, conjugated equine estrogens, liothyronine, ouabain, caspofungin, liotrix, romidepsin, fluvastatin, paclitaxel, docetaxel, cobimetinib, selexipag, ambrisentan, grazoprevir, technetium tc 99m mebrofenin, and parachlorophenol. Should these drugs become necessary during the treatment period, the treating physician should make all efforts to use alternative drugs. However, where it is not feasible or desirable to change to an alternative, the subject must be monitored closely for occurrence of adverse events possibly related to drug accumulation as well as clinical effects of increased exposure, and consideration must be given to reducing the dose.

In addition, the following medications are prohibited during the double-blind treatment period:

d) Systemically administered potent CYP3A4 inhibitors: itraconazole, lopinavir/ritonavir, telaprevir, clarithromycin, ritonavir, ketoconazole, indinavir, conivaptan, and voriconazole.

e) Sensitive CYP3A4 substrates which have a narrow therapeutic range: alfentanyl, astemizole, cisapride, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, systemic sirolimus, systemic tacrolimus, terfenadine. Should fentanyl or alfentanyl use be required during the study, the dose should be carefully titrated.

f) UGT inhibitors and inducers: atazanavir, rifabutin, carbamazepine, phenytoin, oxcarbazepine, nevirapine, methsuximide and phenobarbital.

g) Pruritus medication: rifampicin, naltrexone. Anti-histamines are permitted, if the dose is stable for at least 8 weeks before Screening (Visit 1) and throughout the study.

7 STUDY SCHEDULE

The schedule of assessments table is provided in Appendix A. A schedule of blood sampling, including required volumes, is provided in Appendix B.

7.1 Screening

Visit 1 (Week -4 to -1)

The following assessments and procedures will be performed at Screening:

- Signed informed consent.
- Determination of eligibility.
- Demographics and medical history.
- Height and oral or tympanic temperature.
- Serum pregnancy test for female subjects of childbearing potential.
- Blood sample for viral serology (HIV antibodies 1 and 2, hepatitis B surface antigen and hepatitis C virus antibodies).
- Blood samples for the determination of anti-mitochondrial antibodies (anti-AMA, anti-GP210, anti-SP100, antibodies against the major M2 components [PDC-E2, 2-oxo-glutaric acid dehydrogenase complex]).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Urinalysis (dipstick).
- Pulse rate, SBP and DBP.
- 12-lead ECG.
- Complete physical examination.
- Prior and concomitant medication recording.
- Recording of AEs.

7.2 Enrollment/Baseline

Visit 2 (Day 1)

The following assessments and procedures will be performed at baseline:

- Confirmation of eligibility (not every eligibility assessment needs to be repeated; i.e., confirmatory laboratory and diagnostic tests. Subjects with AEs at baseline may need to be withdrawn in accordance with the eligibility and withdrawal criteria).
- Body weight.
- Urine pregnancy test for female subjects of childbearing potential.
- Optional collection of blood sample for DNA (only for subjects who have signed the additional, specific consent form).
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen, IL-6, CK-18).
- Blood samples for markers of fibrosis (ELF score, collagen fragments).
- Blood samples for optional assessments of immunological markers (IgM, IL-4, IL-12, IL-17A, IFNγ).
- Blood samples for mandatory and optional assessments of bile acid metabolism (serum, C4, total bile acids, FGF-19).
- Blood samples for optional additional biomarkers.
- Blood samples for optional metabolomic studies.
- Transient elastography (FibroScan® or similar technology).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- PBC-40 QoL questionnaire and pruritus VAS.
- Pulse rate, SBP and DBP.
- Symptoms-directed physical examination.
- Concomitant medication recording.
- Randomization.
- Dispense IMP.
- Supervise subject during first self-administration of IMP.
- Recording of AEs.

7.3 Treatment Period

Visit 3 (Week 2 ± 3 Days)

The following assessments and procedures will be performed after 2 weeks of treatment ± 3 days:

- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- Pulse rate, SBP and DBP.
- 12-lead ECG.
- Symptoms-directed physical examination.
- Concomitant medication recording.
- Dispense IMP.
- Blood samples for PK: one pre-dose and two post-dose samples.
- Recording of AEs.
- Record subject's compliance with treatment regimen.

Visit 4 (Week 6 ± 3 Days)

The following assessments and procedures will be performed after 6 weeks of treatment ± 3 days:

- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- Pulse rate, SBP and DBP.
- Symptoms-directed physical examination.
- Concomitant medication recording
- Dispense IMP.
- Recording of AEs.
- Record subject's compliance with treatment regimen.

Visit 5 (Week 12 ± 3 Days)

The following assessments and procedures will be performed after 12 weeks of treatment ± 3 days:

- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen, IL-6, CK-18).
- Blood samples for markers of fibrosis (ELF score, collagen fragments).
- Blood samples for optional assessments of immunological markers (IgM, IL-4, IL-12, IL-17A, IFNγ).
- Blood samples for mandatory and optional assessments of bile acid metabolism (serum, C4, total bile acids, FGF-19).
- Blood samples for optional additional biomarkers.
- Blood samples for optional metabolomic studies.
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- Urinalysis (dipstick).
- PBC-40 QoL questionnaire and pruritus VAS.
- Pulse rate, SBP and DBP.
- 12-lead ECG.
- Symptoms-directed physical examination.
- Concomitant medication recording.
- Dispense IMP.
- Blood samples for PK: one pre-dose and two post-dose samples.
- Recording of AEs
- Record subject's compliance with treatment regimen.

Visit 6 (Week 18 ± 3 Days)

The following assessments and procedures will be performed after 18 weeks of treatment ± 3 days:

- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.

- Pulse rate, SBP and DBP.
- Symptoms-directed physical examination.
- Concomitant medication recording.
- Dispense IMP.
- Blood sample for PK: one post-dose sample.
- Recording of AEs.
- Record subject's compliance with treatment regimen.

Visit 7 (Week 24 ± 3 Days): End of Treatment Visit

The following assessments and procedures will be performed after 24 weeks of treatment (end of treatment) and recorded in the Week 24/End of Treatment eCRF:

- Body weight.
- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen, IL-6, CK-18).
- Blood samples for markers of fibrosis (ELF score, collagen fragments).
- Blood samples for optional assessments of immunological markers (IgM, IL-4, IL-12, IL-17A, IFNγ).
- Blood samples for mandatory and optional assessments of bile acid metabolism (serum, C4, total bile acids, FGF-19).
- Blood samples for optional additional biomarkers.
- Blood samples for optional metabolomic studies.
- Transient elastography (FibroScan® or similar technology).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- Urinalysis (dipstick).
- PBC-40 QoL questionnaire and pruritus VAS.
- Pulse rate, SBP and DBP.
- 12-lead ECG.
- Symptoms-directed physical examination.

- Concomitant medication recording.
- Recording of AEs.
- Record subject's compliance with treatment regimen.

7.4 Final Study Visit (Follow-Up)

Visit 8 (Week 28 ± 3 Days)

The following assessments and procedures will be performed 4 weeks after the end of treatment ± 3 days:

- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen).
- Blood samples for optional additional biomarkers.
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Urinalysis (dipstick).
- Pulse rate, SBP and DBP.
- Complete physical examination.
- Concomitant medication recording.
- Recording of AEs.

7.5 Early Termination Visit

If a subject discontinues treatment earlier than Week 24, the following assessments and procedures should be performed and recorded in the Early Termination eCRF. The subject should return to the clinic within 4 weeks after the Early Termination visit for a Final Study visit as detailed in Section 7.4.

- Body weight.
- Urine pregnancy test for female subjects of childbearing potential.
- Blood samples for markers of inflammation and liver injury (hsCRP, fibrinogen, IL-6, CK-18).
- Blood samples for markers of fibrosis (ELF score, collagen fragments).
- Blood samples for optional assessments of immunological markers (IgM, IL-4, IL-12, IL-17A, IFNγ).
- Blood samples for mandatory and optional assessments of bile acid metabolism (serum, C4, total bile acids, FGF-19).
- Blood samples for optional additional biomarkers.

- Blood samples for optional metabolomic studies.
- Transient elastography (FibroScan® or similar technology).
- Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR.
- Thyroid function tests.
- Urinalysis (dipstick).
- PBC-40 QoL questionnaire and pruritus VAS.
- Pulse rate, SBP and DBP.
- 12-lead ECG.
- Symptoms-directed physical examination.
- Concomitant medication recording.
- Recording of AEs.
- Record subject's compliance with treatment regimen until discontinuation of treatment.

7.6 Unscheduled Visit

All unscheduled study visits, procedures, examinations, clinical laboratory evaluations etc., will be noted in the subject's medical record including:

- Reason for visit/procedure.
- Follow-up.
- Recording of AEs and use of concomitant measures.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

8.1.1 Demographics and Medical History

Demographic and baseline characteristic data will be collected on all subjects at Screening (Visit 1).

Relevant medical history/baseline medical conditions will be recorded.

Prior and concomitant medications history will be recorded (prescription medications, over-the-counter and herbal remedies). Assessment of eligibility at Visits 1 and 2 should include a review of permitted and prohibited medications (see Section 6.6).

8.1.2 Physical Examination

Height will be recorded at Screening (Visit 1). Body weight will be recorded at baseline/Day 1 (Visit 2) and Week 24 (Visit 7).

A complete physical examination will be performed at Screening (Visit 1) and the Week 28 follow-up (Visit 8) and will include: general appearance, skin, head and neck, eyes-ears-nose-throat, lymph node palpation, lungs, chest, abdomen, extremities, and neurological function.

A symptom-directed physical examination will be performed at all other visits. This examination will always be associated with the occurrence of an AE.

In the absence of any AE, the physical examination will be reported “normal” for all items in the eCRF.

8.1.3 Vital Signs

Vitals signs measurements will include:

- Oral or tympanic temperature, at Screening (Visit 1).
- Pulse rate at all visits.
- Blood pressure: SBP and DBP, at all visits.

Supine blood pressure and pulse rate will be measured using an automatic device after the subject has rested comfortably for 5 minutes.

8.1.4 Electrocardiogram

A 12-lead ECG will be performed during Screening (Visit 1) and at Weeks 2, 12, and 24 (Visits 3, 5, and 7, respectively). The measurement will be performed around T_{max} (i.e. 1 to 4 hours post dose).

ECG recordings will be reviewed locally by the Investigator or by a designated cardiologist. ECG parameters including heart rate, QRS axis, PR interval, QRS duration and QT will be recorded.

8.1.5 Transient Elastography (FibroScan® or Similar Technology)

Transient elastography (FibroScan® or similar technology) will be performed at selected centers at baseline/Day 1 (Visit 2) and Week 24 (Visit 7). Absolute and percent change in liver stiffness will be recorded. If transient elastography is not performed, this will not be a major protocol deviation.

8.1.6 Subject Reported Outcomes

PBC-40

All subjects will complete the PBC-40 questionnaire at baseline/Day 1 (Visit 2), Week 12 (Visit 5) and Week 24 (Visit 7).

The PBC-40 is a subject-derived, disease specific QoL measure developed and validated for use in PBC. It is designed for self-completion and takes approximately 45 minutes to complete.

Pruritus VAS

Pruritus will be self-assessed by the subject using a Pruritus VAS at baseline/Day 1 (Visit 2), Week 12 (Visit 5) and Week 24 (Visit 7).

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

The following safety laboratory investigations will be conducted at time points indicated in “Schedule of Assessments” (Appendix A).

The following clinical safety laboratory tests will be performed at each visit:

- Hematology: hematocrit, hemoglobin, absolute and relative reticulocyte counts, red blood cell (RBC) count, white blood cell (WBC) count, differential WBC count, platelet count, absolute neutrophil count, mean cell volume and INR.
- Biochemistry: glucose, total protein, albumin, creatinine, urea, creatine kinase, total cholesterol, triglycerides, sodium, potassium, chloride, ALP, AST, ALT, conjugated and total bilirubin and GGT.

- TSH levels will be tested at each visit, except Visits 1 and 8. Free T4 will be also measured if TSH levels are ≥ 10 mIU/L.

Urinalysis (to be performed at Visits 1, 5, 7 and 8): quantitative test for pH and protein; qualitative tests for glucose, ketones, bilirubin, blood; microscopic examination of the sediment.

Viral serology will be performed at Screening (Visit 1): HIV antibodies (1 and 2), hepatitis B surface antigen and hepatitis C virus antibodies.

Determination of AMA will be performed at Screening (Visit 1): AMA titer, or if AMA is negative or in low titer (<1:80) PBC specific antibodies (anti-GP210 and/or anti-SP100 and/or antibodies against the major M2 components [PDC-E2, 2-oxo-glutaric acid dehydrogenase complex]).

Female subjects of child-bearing potential must have a negative serum pregnancy test documented at Screening (Visit 1). A negative urine pregnancy test must be done prior to study intervention at baseline/Day 1 (Visit 2) and all subsequent visits and the results must be available prior to dispensing of IMP.

8.2.2 Pharmacokinetics

For the PK assessments, venous blood samples of 2 mL each taken by venipuncture at the time points listed in Table 6.

Table 6 Pharmacokinetic Time Points

Visit	Pre-dose time point	Post-dose time points
Week 2 ± 3 Days (Visit 3)	One 2 mL sample taken before subject takes the morning dose	• One 2 mL sample taken 1-2 hours after morning dose • One 2 mL sample taken before subject leaves clinic but no earlier than 2 hours after first post-dose sample
Week 12 ± 3 Days (Visit 5)	One 2 mL sample taken before subject takes the morning dose	• One 2 mL sample taken 1-2 hours after morning dose • One 2 mL sample taken before subject leaves clinic but no earlier than 2 hours after first post-dose sample
Week 18 ± 3 Days (Visit 6)	N/A	One 2 mL sample taken before subject leaves clinic at 3-4 hours after morning dose

Plasma samples will be analyzed for the determination of concentrations of GKT137831 and its main active metabolite, GKT138184, using a validated bioanalytical method.

Detailed information about the blood sample collection, processing, handling, storage, and shipping is provided in the Laboratory Manual.

8.2.3 Pharmacogenetics

Genetic and pharmacogenetic research may be conducted on the DNA samples collected during the study from subjects who sign an optional, additional informed consent. A 5 mL venous blood sample will be collected into a suitable tube at baseline/Day 1 (Visit 2) pre-dose for genotyping purposes.

Sample labels will include:

- Study Number.
- Genotyping.
- Subject ID number.
- Collection date (dd/mm/yyyy).
- Visit 2 pre-dose.

Detailed information about the blood sample collection, processing, handling, storage, and shipping is provided in the Laboratory Manual.

8.2.4 Pharmacodynamics

All PD samples are collected pre-dose and in fasting condition.

Venous blood samples (4 mL at each sampling time point) will be collected for markers of inflammation and liver injury (hsCRP, fibrinogen) at baseline/Day 1 (Visit 2), and Weeks 2, 6, 12, 18, 24 and 28 (Visits 3 – 8).

Venous blood samples (4 mL at each sampling time point) will be collected for optional markers of inflammation and liver injury (IL-6, CK-18) at baseline/Day 1 (Visit 2), Weeks 12 and 24 (Visits 5 and 7).

Venous blood samples (4 mL at each sampling time point) will be collected for markers of fibrosis (ELF score and collagen fragments) at baseline/Day 1 (Visit 2), at Weeks 12 and 24 (Visits 5 and 7).

Venous blood samples (1.5 mL at each sampling time point) will be collected for optional metabolomics studies, in fasting condition at baseline/Day 1 (Visit 2), and at Weeks 12 and 24 (Visits 5 and 7).

Venous blood samples (4 mL at each sampling time point) will be collected for mandatory and optional markers of cholestasis (Serum C4, total bile acids, and FGF 19) at baseline/Day 1 (Visit 2), Weeks 12 and 24 (Visits 5 and 7).

Venous blood samples (3 mL at each sampling time point) will be collected for optional immunological markers (IgM, IL4, IL12, IL1-7A, interferon γ) at baseline/Day 1 (Visit 2), Weeks 12 and 24 (Visits 5 and 7).

Venous blood samples (3 mL at each sampling time point) will be collected for optional biomarkers of interest. These samples will be collected at baseline/Day 1 (Visit 2), Weeks 12, 24 and 28 (Visits 5, 7 and 8). The identity of these exploratory markers will be defined in response to findings described in the literature or obtained in this study. These markers will not have diagnostic or prognostic value and will not have an established normal range according to WHO.

Detailed information about the blood sample collection, processing, handling, storage, and shipping is provided in the Laboratory Manual.

9 ASSESSMENT OF SAFETY

9.1 Safety Parameters

The safety of GKT137831 will be assessed through the recording, reporting and analyzing of baseline medical conditions, AEs, general physical examination, laboratory tests, 12-lead ECGs, and vital signs data. The timing and frequency of safety assessments are described in Section 8.

9.2 Adverse Events

9.2.1 Definition of Adverse Event

An AE is defined as any untoward medical occurrence in the form of signs, symptoms, abnormal laboratory findings, or disease that emerges or worsens relative to baseline during a clinical study with an IMP, regardless of causal relationship and even if no IMP has been administered.

Unchanged/stable pre-existing, chronic medical conditions present at baseline, or those medical conditions related to the underlying disease whose changes during the study that are consistent with natural disease progression are NOT considered as AEs and should not be recorded in the AE pages of the eCRF unless a worsening has occurred.

9.2.1.1 Severity of Adverse Events

AEs will be graded according to the CTCAE v4 grading system. The CTCAE v4 grading criteria must be used when available for an AE. Only when CTCAE v4 grading criteria are not available for a specific AE, will the Investigator assess the severity of the AE based on the following definitions:

- Grade 1 (mild): the subject is aware of the event or symptom, but the event or symptom is easily tolerated.
- Grade 2 (moderate): the subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.
- Grade 3 (severe): the subject is unable to carry out usual activities due to significant impairment of functioning.
- Grade 4 (life-threatening): the subject’s life is at risk from the event.

9.2.1.2 Causality Assessment

The Investigator will determine whether or not, in his/her opinion, the AEs are related to the IMP according to the following definitions:

- Unrelated: due to extraneous causes and does not meet criteria listed under unlikely, possible or probable.

- Unlikely (or improbable): relationship not likely according to present knowledge. Does not follow a reasonable temporal sequence from administration. May have been produced by the subject's clinical state or by environmental factors or other therapies administered.
- Possible: follows a reasonable temporal sequence from administration: possibility that the AE may have been caused by the IMP but may also have been produced by the subject's clinical state or by environmental factors or other therapies administered.
- Probable: clear-cut temporal association with improvement on cessation of IMP or reduction in dose. Reappears upon re-challenge. Follows a known pattern of response to IMP.

9.2.1.3 Abnormal Laboratory Values/and Other Objective Measurements

Abnormal laboratory findings and other objective measurements such as vital signs and ECGs, should NOT routinely be captured and reported as AEs as they will be collected and analyzed separately in the eCRF. However, abnormal laboratory findings and other objective measurements which (i) meet the criteria for a SAE (see Section 9.3.1), or (ii) result in discontinuation of the IMP or in treatment being withheld (see Sections 5.6.4 and 6.3, respectively), or (iii) require medical intervention should be captured and reported in the AE pages of the eCRF.

If reporting an abnormal laboratory finding in the AE pages of the eCRF, a clinical diagnosis should be recorded rather than the abnormal value itself, if available (for example "anemia" rather than "decreased RBC count" or "hemoglobin = 10.5 g/dl").

9.2.2 Eliciting Adverse Events

AEs will be obtained by the Investigator at scheduled or unscheduled study visits, following physical examination, based on information spontaneously provided by the subject and/or through questioning.

To elicit AEs, simple questions with minimal connotations should be used as the initial questions at all evaluation points during the study. For example:

- How have you felt since your last visit?
- Have you had any health problems since you were here last?

In the case that a subject was seen by a health care professional other than the Investigator (e.g., at a different institution) concerning an AE, every effort should be made by the Investigator to contact the treating physician in a timely manner in order to obtain all necessary information and report the event appropriately.

9.2.3 Recording Adverse Events

As quality and precision of acquired AE data is of key importance, the Investigator should use the AE definitions provided in above sections and observe the following guidelines when completing the AE pages of the eCRF:

- Whenever possible, recognized medical terms should be used to describe AEs rather than colloquialisms (for example, 'influenza' rather than 'flu'), and abbreviations should be avoided.
- AEs should be described using a specific clinical diagnosis rather than component signs or symptoms, if this is available (for example, 'congestive heart failure' rather than 'dyspnea, rales and cyanosis'). However, signs and symptoms considered unrelated to an identified disease or syndrome should be reported as individual AEs in the eCRF.
- AEs occurring secondary to other events (e.g., sequelae or complications) should be identified by the primary cause. A primary AE, if clearly identifiable, generally represents the most accurate clinical term to record in the AE pages of the eCRF.

Additional guidance can be found in the eCRF completion conventions provided by the Sponsor.

For the purposes of this study, any detrimental change in the subject's condition, after signing the informed consent form (ICF) and up to completion of the 28-day follow-up period after the last administration of IMP, should be considered an AE. AEs should be followed-up in accordance with the procedures described in Section 9.2.4.

9.2.4 Follow-up of Adverse Events

The primary objective of the post-treatment period is to check for any withdrawal and/or residual effects of GKT137831 which manifest after discontinuation of IMP administration. Reporting of AEs during the post-treatment period is defined as follows:

- All AEs which occur within 28 days from the last administration of IMP will be recorded in the AE pages of the eCRF as defined above.
- All ongoing AEs considered at least possibly related to the IMP will be followed until resolution or until the Investigator assesses them as 'chronic' or 'stable'.
- If the Investigator becomes aware of a SAE, including death, after the 28 days post-treatment period and the SAE is considered by the Investigator to be at least possibly related to the IMP, it will be reported to the designated CRO safety group in accordance with Section 9.3.2.

Taking the above post-treatment safety surveillance reporting requirements into account, AE follow-up information data will be recorded in the AE pages of the eCRF up until Last Subject Last Visit.

After Last Subject Last Visit and prior to database lock, the status of all outstanding ongoing AEs will be checked and any up-to-date change made in the eCRF. At that stage, as considered appropriate by the Sponsor, follow-up information will continue to be requested from the Investigator on specific

AEs (and in any case all SAEs considered at least possibly related by the Investigator) although related details will not be recorded in the eCRF beyond database lock.

9.3 Serious Adverse Events

9.3.1 Definition of Serious Adverse Event

A SAE is defined as an AE which meets one of the following criteria:

- Results in death.
- Is life-threatening (defined as a subject at immediate risk of death at the time of the event).
- An event requiring inpatient hospitalization or prolongation of existing hospitalization.

Note: In general, hospitalization signifies that a person has been detained (usually overnight) at a hospital or emergency ward for observation and/or treatment. Emergency room visits which do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious, medically important event.

- Results in a persistent or significant disability/incapacity.

Note: The term significant disability/incapacity means a substantial disruption of a person's ability to conduct normal life functions, i.e., the AE resulted in a significant, persistent or permanent change, impairment, damage or disruption in the subject's body function/structure, physical activities and/or quality of life.

- Is a congenital anomaly or birth defect.
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the above criteria. An AE of severe intensity might not necessarily be considered serious. For example, persistent nausea for several hours may be considered severe, but not a SAE. Conversely, a stroke resulting in only a limited degree of disability may be considered mild, but would be a SAE.

All SAEs will be:

- Recorded in the eCRF from the time of signed informed consent until 28 days after the last dose of IMP, regardless of suspected relationship to IMP.

- Recorded on the appropriate SAE Report Form and reported in accordance with the procedures described in Section 9.3.2.
- Followed until resolution, stabilization or until determined to be chronic by the Investigator.
- Reviewed and evaluated by the Investigator.

9.3.2 Reporting of Serious Adverse Events

Any SAE occurring in a subject who has signed the informed consent and until 28 days after the last administration of IMP must be reported by the Investigator to the designated CRO safety group (Cmed) **within 24 hours of first awareness** even if the SAE does not appear to be associated with the IMP. Any SAEs experienced after this period should be reported to the designated CRO designee safety group if the investigator has assessed that the SAE is reasonably related to IMP administration.

Information about the SAE (either initial or follow-up) should be collected and recorded on the paper Serious Adverse Event Report Form. A causality assessment must be provided at the time of reporting. The form should be completed in English and signed and dated by the investigator.

SAEs should be reported by emailing or faxing a copy of the SAE Report form plus other relevant information to the designated CRO safety group. The SAE may be reported by telephone; however, this should be followed up within 24 hours with a copy of the SAE Report form. Additionally, it may be necessary for the designated CRO safety group to communicate with the Investigator if additional information is required.

During both business and non-business hours, the email address, telephone and fax numbers listed below should be used to notify the designated CRO safety group:

Reportable Events Hotline:
Cmed Clinical Services
Email: *sae@cmedresearch.com*
24 Hour Phone: 0044 (0)1403 758462
US Toll-Free Phone: 1 866 966 8429
Fax: 0044 (0)1403 330459
US Toll-Free Fax: 1 866 966 2970

The SAE Report Form must be completed and forwarded via email or facsimile to the designated CRO safety group using the email address or fax number listed above within 24 hours of becoming aware of the event.

All additional follow-up evaluations must be reported within 24 hours to the designated CRO safety group. All SAEs will be followed until resolution, stabilization or the Investigator determines the event to be chronic.

The Sponsor is responsible for complying with applicable regulatory reporting requirements for SAEs and, where necessary, for ensuring the relevant authorities are notified (see Section 9.4). The Investigator will ensure the appropriate IRBs/IECs are notified of the SAE in accordance with ICH GCP and local regulatory requirements.

9.4 Regulatory Reporting of Serious, Unexpected, Adverse Reactions (SUSARs)

Following notification from the Investigator, the Sponsor and/or it's designee (CRO) will report events which are both serious and unexpected (not previously described in the reference safety information in the approved IB) and that are associated with the IMP to the FDA, EMA, other concerned Regulatory Authorities, IRB/IECs and investigators within the required timelines as specified in 21 Code of Federal Regulations (CFR) Part 312.32, EU Directive 2001/20/EC, the European Commission's "Detailed guidance on the collection, verification, and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use" (CT-3, June 2011), ICH GCP Guidelines and other applicable national regulatory requirements. Typically, fatal and life-threatening events must be reported within 7 calendar days and all other SAEs must be notified in within 15 calendar days. All serious events designated as not related to the IMP, will be reported to the concerned Regulatory Authorities, and IRB/IECs at least annually in a summary format.

Additionally, events may occur during a clinical trial which do not fall within the definition of a SUSAR and thus are not subject to the reporting requirements for SUSARs, even though they may be relevant in terms of subject safety. Examples are new events related to the conduct of a trial or the development of an IMP likely to affect the safety of subjects such as:

- A SAE which could be associated with the trial procedures and which could modify the conduct of the trial.
- A significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease.
- A major safety finding from a newly completed animal study (such as carcinogenicity).
- A temporary halt of a trial for safety reasons if the trial is conducted with the same IMP in another country by the same Sponsor, recommendations of the SMB, if any, where relevant for the safety of subjects.

These events/observations are not to be reported as SUSARs, but they might require other action, such as urgent safety measures and their notification, substantial amendments, or early termination of the trial, and shall be reported in accordance with applicable local regulations and guidelines.

9.5 Drug Induced Liver Injury

If a study subject, who has been exposed to the IMP, presents signs or symptoms of DILI, a close monitoring must be initiated and documented by the investigator. The IMP must be discontinued until re-testing is performed, additional investigations completed and a diagnosis made. If DILI is confirmed or suspected by the investigator, the investigator will complete an Expedited Liver Assessment Report form (ELAR) and submit it to Sponsor in the same timelines as a SAE (see Section 9.3.2. The ELAR form must be completed and forwarded via email or facsimile to the designated CRO safety group using the email address or fax number listed in Section 9.3.2 within 24 hours of the Investigator becoming aware of the confirmed or suspected DILI event.

9.6 Pregnancy

If a female study subject who has been exposed to the IMP becomes pregnant, the course and outcome of the pregnancy should be monitored and documented. The IMP must be discontinued. If a female partner of a male study subject who has been exposed to the IMP becomes pregnant, and the subject provides this information, then the pregnancy will be documented, based on information provided by the subject. Any pregnancy (either female study participants or female partners of male study participants) must be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Consent to report information regarding pregnancy outcome should be obtained from the female partners of male study participants. The study treatment must be discontinued for pregnant participants.

Although pregnancy itself is not considered as an AE, all pregnancies occurring inadvertently during the study pre-treatment, treatment and post-treatment period must be reported in the same timelines as a SAE, but by using the Clinical Trial Pregnancy Report Form. The form must be completed and forwarded via email or facsimile to the designated CRO safety group using the email address or fax number listed in Section 9.3.2 within 24 hours of the Investigator becoming aware of the pregnancy.

If the outcome or course of the pregnancy involves a SAE (e.g., a congenital anomaly) then the SAE form should be completed in addition to the updated Clinical Trial Pregnancy Report Form. Spontaneous abortions and congenital birth defects should always be reported as SAEs (see Section 9.3).

9.7 Safety Monitoring Board

In order to ensure subject safety, a SMB will conduct periodic, scheduled and unscheduled reviews of subject data while the study is in progress. The role and responsibilities of the SMB will be outlined in detail in a separate SMB charter.

The predefined triggers, scope, and process for unscheduled SMB meetings will be outlined in the SMB charter. Briefly, at any time, the SMB, Study Director or Sponsor may request an unscheduled SMB review of the study data based on concern for subject safety. In addition, unscheduled SMB

meetings will take place if predefined events occur, including any death or immediately life-threatening condition, or a permanent interruption of IMP administration due to DILI or anemia.

The SMB will receive outputs of the unblinded eCRF and laboratory data, and adjudicate on subject status changes and dosing decisions (where appropriate). The data will include, but is not limited to, demographics, subject enrolment, baseline characteristics, AE data, SAE data (by severity and causality), laboratory data including PK, dose adjustments, protocol adherence, and subject withdrawals. The SMB will evaluate the progress of the study, assess data quality and timeliness, participant recruitment, accrual and retention, and participant risk versus benefit. In addition the SMB will monitor external factors relevant to the study. For example, scientific and therapeutic developments which may affect participant safety or ethical status. Based on the observed benefits or adverse effects, the SMB will make recommendations to the Sponsor concerning continuation, termination or modifications of the study.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

The testing assumption for the primary endpoint, for each dose separately vs placebo, is:

$$H_0 : \mu_{G1} = \mu_P,\ \mu_{G2} = \mu_P$$
$$H_A : \mu_{G1} \neq \mu_P \quad \text{or} \quad \mu_{G2} \neq \mu_P$$

Where:

μ_{G1}, μ_{G2} Mean percent change from baseline in serum GGT after 24 weeks in the active GKT137831 group.

μ_P Mean percent change from baseline in serum GGT after 24 weeks in the Placebo group.

The null hypothesis is that there is no difference in efficacy between the two doses of GKT137831 and placebo. The alternative hypothesis is that there is a difference.

10.2 Sample Size Considerations

A sample size of 34 randomized and treated subjects within each treatment group with an overall sample size of 102 subjects will have 80% power to detect a 28% difference in the means of the percent change from baseline in serum GGT. A Wilcoxon Mann-Whitney test has been used for the sample size estimate because this represents the worst-case scenario with regards to statistical power. A standard deviation of 30 for the active GKT137831 group and a standard deviation of 40 for the placebo group have been assumed based on a recent phase 3 clinical trial of OCA [65].

An overall two-sided Type I error of 5% has been considered for the sample size estimate. The sample size estimate allows for one interim analysis using the O'Brien-Fleming spending function. This gives an alpha spend of 0.00305 at the interim analysis stage. The Hochberg method will be used to adjust the alpha level for multiple comparisons, therefore the first dose level comparison at the final analysis will be tested against an alpha level of 0.04695 and the second dose level will be tested against an alpha level of 0.023475. The alpha level of 0.023475 has been used for the sample size calculation.

The sample size calculation assumes all subjects, including withdrawals will be included in the analysis. Hence there has been no adjustment for dropouts.

10.3 Planned Interim Analyses

An interim analysis is planned once 90 subjects have completed their Week 6 visit. Only data collected for the first 90 randomized subjects, prior to and including the date of the Week 6 visit for the 90th randomized subject, will be included in the interim analysis. The purpose of the interim analysis is to support decision making with regards to the further development of GKT137831. It is not intended to amend the study protocol or stop the trial due to futility or overwhelming efficacy.

The interim analysis will include key efficacy data and key safety information such as AEs and supportive analyses of the secondary endpoints. The specific contents of the interim analysis will be documented in the SAP. The sample size has been adjusted for the interim analysis using an O'Brien-Fleming alpha spending function which allows for an alpha spend of 0.00305 for the analysis of the primary endpoint.

The interim analysis will be conducted by the unblinded SMB statistician, who has no involvement with study conduct. The interim analysis outputs will be communicated to the SMB and to a predefined disclosure committee, to aid decision-making with regards to the further development of GKT137831. However, to maintain the integrity of the trial data, no unblinding information for individual subjects will be shared outside of the SMB. Detailed communication flow will be outlined in the SMB charter and SAP.

In addition to the interim analysis described above a SMB will oversee the safety of the participating subjects as described in Section 9.7.

10.4 Analysis Methods

10.4.1 Analysis Populations

The **Intent-To-Treat (ITT)** population will include all randomized subjects who receive at least one dose of IMP or placebo. The ITT population will be analyzed by the randomized treatment and will be used as the primary population for all analyses of efficacy.

The **Safety** population will be used for the analysis of all safety data and will include all subjects who received at least one dose of IMP or placebo, irrespective of whether they were randomized. The Safety population will be analyzed by the treatment received.

The **Per Protocol (PP)** population will be a subset of the ITT population and will include all subjects without a major protocol deviation. Protocol deviations will be defined with the classification of subjects excluded from analysis populations prior to unblinding at end of study.

The PP population will be analyzed by the randomized treatment. This population will be for supportive analysis of the primary endpoint and secondary endpoints where deemed relevant.

Major protocol deviations will be summarized by treatment group. All protocol deviations deemed important even if not leading to subject exclusion from the PP population will be listed.

The **Pharmacokinetic (PK)** population will be used for the analysis of pharmacokinetic data and will include all subjects who received at least one dose of IMP, who have at least one valid PK measurement and who have no major protocol deviations relating to PK data. The PK population will be analyzed by the treatment received.

10.4.2 Definition of Analysis Endpoints: Primary, Secondary, Tertiary, Exploratory, Safety and Pharmacokinetics

10.4.2.1 Primary Efficacy Endpoint

The percent change from baseline to Week 24 (Visit 7) in serum GGT

10.4.2.2 Secondary Efficacy Endpoints

- Absolute and percent change in serum GGT from baseline to each assessment.
- Absolute change in ELF score from baseline to Weeks 12 and 24.
- Absolute and percent change in serum ALP from baseline to each assessment.
- Absolute and percent change in serum levels of hsCRP, and fibrinogen, from baseline to each assessment.
- Absolute and percent change in serum ALT, AST, and conjugated and total bilirubin, from baseline to each assessment.
- Absolute and percent change in the FIB-4 and APRI scores, from baseline to each assessment (FIB-4: age (years) x AST (IU/L)/(platelet count (10^9/L) x (ALT (IU/L)1⁄2, APRI: AST (IU/L)/ upper normal limit AST)x100/platelet count (10^9/L).
- Absolute and percent change in liver stiffness as assessed by transient elastography (FibroScan® or similar technology), from baseline to Week 24, in subjects with values at baseline and Week 24.
- Absolute and percent change in serum levels of collagen fragments indicative of collagen formation and degradation, from baseline to Weeks 12 and 24.
- Absolute and percent change in total bile acids score from baseline to Weeks 12 and 24.

10.4.2.3 Tertiary Efficacy Endpoints

- Absolute and percent change in Quality of Life and Pruritus scores based on the PBC-40 and Pruritus VAS, from baseline to Weeks 12 and 24.
- Proportion of subjects achieving a 15, 20, 30 and 40% reduction in serum ALP from baseline to each assessment.
- Proportion of subjects who meet the definition of PBC responder criteria applying the Paris I, Toronto I, Toronto II, Toronto III, Toronto IV, Mayo II and Barcelona disease prognostic risk criteria at Weeks 12 and 24.

10.4.2.4 Exploratory Endpoints

- Optionally, absolute and percent change in serum C4, and FGF19 from baseline to Weeks 12 and 24.
- Optionally, absolute and percent change in serum IL-6 and CK-18, from baseline to Weeks 12 and 24.
- Optionally, absolute and percent change in serum IgM, IL-4, IL-12, IL-17A, and interferon γ, from baseline to Weeks 12 and 24.
- Optionally, assessment of metabolomics signatures.
- Optionally, assessment of additional biomarkers of interest.

10.4.2.5 Safety Endpoints

- Treatment-Emergent Adverse Events (TEAEs) defined as any AE occurring after the first intake of IMP.
- Hematology: Blood levels and shift changes from baseline of hematocrit, hemoglobin, absolute and relative reticulocyte counts, RBC count, WBC count, differential WBC count, platelet count, absolute neutrophil count, mean cell volume and INR.
- Biochemistry: Blood levels and shift changes from baseline of glucose, total protein, albumin, creatinine, urea, creatine kinase, total cholesterol, triglycerides, sodium, potassium, chloride, ALP, AST, ALT, conjugated and total bilirubin and GGT.
- Urinalysis: quantitative test for pH and protein; qualitative tests for glucose, ketones, bilirubin, blood; microscopic examination of the sediment.
- TSH.
- Pulse rate, SBP and DBP.
- Body weight.
- 12-lead ECG.

10.4.2.6 Pharmacokinetic Endpoints

- Plasma concentration of GKT137831.
- Plasma concentration of GKT138184, the main phase 1 metabolite of GKT137831.

10.4.3 Statistical Methodology

10.4.3.1 Demography and Disease History

Demography variables and key baseline disease characteristic variables such as baseline ALP, baseline UDCA dose and time since diagnosis will be summarized by treatment group using the ITT population.

10.4.3.2 Primary Efficacy Analysis

The mean of all assessments, including repeat assessments, prior to first dose will be considered as the baseline value for the analysis.

Due to the small sample size the primary analysis will be conducted using a stepwise approach. The percent change from baseline to Week 24 in serum GGT will be analyzed using an Analysis of Covariance (ANCOVA) with treatment and disease severity as fixed effects, and baseline GGT as a continuous covariate. If the normality assumption for the analysis is not met then the percent change from baseline in GGT at Week 24 will be analyzed non-parametrically through a stratified Wilcoxon Mann-Whitney (van Elteren) test. The normality assumption will be assessed through the examination of diagnostic residual plots. Further details and assumption checking will be provided in the SAP.

The difference between each dose of GKT137831 and placebo will be calculated, along with 95% and 97.5% confidence intervals of the difference (to account for Hochberg adjustment).

The primary analysis will be performed using the ITT population and a sensitivity analysis will be done using the PP population. If the Wilcoxon Mann-Whitney test is used, a subgroup analysis for disease severity will also be performed.

10.4.3.3 Second Efficacy Analysis

Any analyses of the secondary efficacy endpoints should be interpreted with care. The study has not been powered for the interpretation of these endpoints. Inferential statistical analyses performed on the secondary endpoints are included to aid interpretation and should not be considered as an alternative to the primary analysis for determining efficacy. All analyses will be performed using the ITT population. Sensitivity analyses using the PP population may also be performed on secondary endpoints; these will be specified in the SAP.

The difference in the percent change from baseline in serum GGT between each dose level of GKT137831 and placebo will be estimated for all scheduled visits using a repeated measures analysis of covariance model with treatment, visit and disease severity as fixed effects, the baseline value as a continuous covariate and the interaction effect between treatment and visit. Should the repeated measures analysis not converge or the normality assumption fail, data from each visit will be compared individually using the same method as the primary analysis.

Descriptive summary statistics by treatment group and visit will be presented for the change from baseline and absolute values of ELF score. The difference in the change from baseline in ELF score between each dose level of GKT137831 and placebo will be estimated for all scheduled visits using the same methodology as for serum GGT.

Serum ALP will be summarized in the same way and analyzed using the same methodology as for serum GGT.

In addition the proportion of subjects achieving a 15, 20, 30, and 40% reduction in serum ALP will be tabulated.

Summary statistics will also be presented for the percent change from baseline, change from baseline and absolute values for all continuous secondary efficacy endpoints. The change from baseline in liver function tests other than ALP may also be analyzed in an exploratory manner using the same statistical methodology as for the serum GGT.

With regard to the PBC-40, analysis is by domain, with the scoring explained in the coded-PBC-40. Data should be considered by domain rather than in terms of a cumulative PBC-40 score. If data are missing from a domain (typically missed or duplicated answers) the whole domain should be discarded if <50% of items are completed. If >50% of responses are present then the median value for the completed items in the domain should be ascribed to the missing item.

The number and percentage of subjects belonging to each group for categorical efficacy endpoints will be presented by visit and treatment group. Such endpoints include but are not limited to, responders to the Paris I, Toronto I, Toronto II, Toronto III, Toronto IV, Mayo II, and Barcelona disease prognostic risk criteria for PBC.

All descriptive statistics will be presented overall, by treatment severity and by any other appropriate subgroups as defined in the SAP provided sufficient subject numbers are available within each subgroup level, within each treatment group.

10.4.3.4 Safety

All analyses of safety endpoints will be analyzed using the safety population.

Adverse Events:

The number and percentage of subjects experiencing an AE will be summarized by treatment group as per the following criteria, both overall and by system organ class and preferred term:

- TEAEs;
- Related TEAEs;
- Serious TEAEs;
- Related and serious TEAEs;

- TEAEs leading to discontinuation.

TEAEs will be defined as AEs that start on or after the date of the first dose of IMP. In addition, summaries of the number and percentage of subjects experiencing an AE by system organ class, preferred term and maximum severity will be produced as per the following criteria:

- TEAEs;
- Related TEAEs.

Clinical Laboratory Tests:

The number and percentage of abnormal biochemical (including TSH), hematological and urinalysis laboratory results will be presented by treatment group, laboratory parameter, planned visit and severity, where applicable. For dipstick parameters with a categorical response any positive results will be considered abnormal. The CTCAE criteria will be used to determine severity. Shift tables for the change from baseline to each planned visit will also be presented. In addition descriptive summary statistics will be produced for all biochemical (including TSH) and hematological lab parameters which are not considered efficacy endpoints. For the calculation of summary statistics all laboratory results will be converted to SI units. Differential counts will be presented both as absolute values and as percentages of white blood cells.

Prior and Concomitant Medications:

The number and percentage of subjects taking a medication will be summarized by treatment group, anatomical therapeutic chemical level 2 and preferred term. Summaries will be produced for any medications started prior to first dose (and ongoing at baseline) and medications started on or after the date of first dose.

Vital Signs and Body Weight:

Pulse rate, SBP, DBP and body weight will be summarized using descriptive statistics by treatment group and planned visit.

Both the absolute values and the change from baseline will be considered.

12-lead ECG:

Heart rate, QT interval (both uncorrected and corrected using Bazett's and Fredericia's formulas), will be summarized using descriptive statistics by treatment group and planned visit.

Both the absolute values and the change from baseline will be considered.

Adverse Events of Special Interest:

Potential effect of GKT137831 on arterial blood pressure and its clinical relevance will be evaluated. Systolic and diastolic blood pressure values will be examined together with temporally associated blood pressure related events including adverse events and serious adverse events, changes in study drug administration and other related changes in concomitant medications. Medical history and other possible contributing risk factors will also be assessed. Results will be presented by treatment group and planned visit.

10.4.3.5 Pharmacokinetics

Plasma concentrations of GKT137831 and its main phase 1 metabolite GKT138184 will be summarized using descriptive statistics by dose level and planned visit. Any plasma concentrations below the limit of quantification will be considered as 0 for the calculation of the summary statistics. The actual sampling time will be presented in listings. Pre-dose samples collected post dose will automatically be flagged for exclusion in the summary statistics.

10.4.3.6 Pharmacokinetic/Pharmacodynamic Analysis

Samples will be analyzed to determine plasma drug concentrations and thus to aid investigation of any PK/PD relationship with PD and/or efficacy endpoints, and optionally for the relationships between plasma drug concentrations and pharmacogenomics data.

A population PK model describing the plasma concentrations of GKT137831 and GKT138184 will be developed using non-linear mixed-effects modelling. Relationships between drug concentrations/ exposure measures and selected PD and/or therapeutic efficacy endpoints in the same subject will be graphically explored and formal PK/PD (exposure-response) analyses may be performed. A separate Modeling and Simulation Analysis Plan (MSAP) for the PK/PD modeling describing the general approach to be taken will be finalized prior to database lock. The actual execution of any PK/PD modeling will depend upon the data, and full details of this will be provided in a separate report, which will be appended to the clinical study report (CSR).

10.4.3.7 Missing Data/Outliers/Dropout Considerations

For the primary analysis all subjects will be included with missing data imputed using method MAR (missing at random) or MNAR (missing not at random), as appropriate. This same approach will be used regardless of whether an ANCOVA or Wilcoxon Mann-Whitney test is used to analyze the primary endpoint. More details, including sensitivity analyses, will be provided in the SAP. The complete case approach will be used for the analysis of categorical variables using a worst-case imputation strategy. For all other analyses and summaries no imputation will be performed.

10.4.3.8 Other Statistical Analysis Considerations

All safety and efficacy data collected by visit will be summarized regardless of the visit window. For ECG, vital signs, laboratory results and efficacy assessments if there is more than one result

recorded for the same visit the non-missing value closest (if it is a planned, repeat or unscheduled assessment) to the planned day will be used in the summary tables and will be flagged into the corresponding listing. If there is more than one value closest to the planned day, the latest value will be used if the assessment was performed prior to the last dose of IMP.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site shall maintain appropriate medical and research records for this trial, in compliance with ICH GCP and regulatory and institutional requirements for the protection of subject confidentiality. The Investigator/institution shall provide direct access to source data/documents for study related monitoring, audits, IRB/IEC review and regulatory inspection.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, recorded audio tapes of counseling sessions, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

The eCRF is not a source document.

12 QUALITY CONTROL AND QUALITY ASSURANCE

12.1 Conduct of the Study

The Sponsor and Cmed shall implement and maintain quality control (QC) and quality assurance (QA) procedures with written standard operating procedures (SOPs) to ensure the study is conducted and data are generated, documented and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with USA FDA regulations (CFR, Sections 312.50 and 312.56) and with ICH GCP.

The Investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by the IRB/IEC and the concerned Regulatory Authorities, when applicable, except when necessary to eliminate immediate hazards to the subject or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the subject having to be withdrawn from the study or render that subject non-evaluable.

All participating sites are required to have procedures in place for assuring the quality of the research being conducted, including, but not limited to:

- How data will be evaluated for compliance with the protocol and for accuracy in relation to source documents.
- The documents to be reviewed (e.g., eCRFs, clinic notes, product accountability), who is responsible, and the frequency for reviews.
- Methods of training for staff, and methods of tracking such training.

12.2 Study Monitoring

During the course of the study, a CRA will conduct routine site visits to review protocol compliance, compare eCRF entries with individual subject's original source documents (accessed by the Investigator), assess product accountability and ensure the study is conducted according to applicable regulatory requirements. The review of the subject's original medical records shall be performed in a manner which ensures subject confidentiality is maintained.

The Investigator shall permit the CRA to review study data as frequently as deemed necessary to ensure that data are recorded in an adequate manner and that protocol adherence is satisfactory.

The Investigator may not enroll subjects into the study until such time that an initiation visit, or with the agreement of the Sponsor, attendance at the Investigator meeting, has been performed by the CRA to conduct a detailed training of the protocol and eCRF.

13 ETHICS

13.1 Ethical Standard

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for GCP, with applicable local regulations (including European Directive 2001/20/EC, US CFR Title 21), and with the ethical principles laid down in the Declaration of Helsinki.

Records that may reveal the identities of subjects must be well protected, with consideration given to confidentiality and the right to privacy.

Each Principal Investigator will complete, sign and date the FDA 1572 form prior to conducting any study-related activities. Each Principal Investigator and Sub-Investigator will complete, sign and date the Financial Disclosure Form to declare any financial or other competing interests. Participating investigators will receive protocol training either at the Site Initiation Visit (SIV) or via attendance at an Investigator Meeting.

13.2 Institutional Review Board/Independent Ethics Committee

Before initiating the study, the Investigator/institution should obtain approval/favorable opinion from the IRB/IEC for the trial protocol, written ICF, ICF updates, subject recruitment procedures (e.g., advertisements) and any other written information to be provided to subjects. The IRB/IEC shall be appropriately constituted and perform its functions in accordance with FDA, EU, ICH GCP, and local requirements as applicable.

Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to the CRA, auditors, the Sponsor's QA representatives, designated agents of the Sponsor, IRBs/IECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform the Sponsor immediately that this request has been made.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the subject's agreeing to participate in the study and continues throughout the subject's study participation. Written documentation of informed consent is required prior to any performance of study procedures.

A site-specific, IRB/IEC-approved ICF, describing in detail the study treatments and procedures, visit schedule, restrictions and risks and possible benefits, will be given to the subject during a clinic visit. The subject will be asked to read and review the document. The Investigator will explain the research study to the subject and answer any questions that may arise. The subject should have the

opportunity to discuss the study with others if they wish and should be given adequate time to consider their decision before to agreeing to participate. If the subject agrees to participate, he/she will sign and date the ICF with the Investigator. A copy of the signed informed consent document will be given to the subject to keep.

The subject may withdraw consent at any time throughout the course of the trial without affecting their legal rights or incurring loss of benefits to which they are otherwise entitled. The rights and welfare of the subject will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study, or by withdrawal of consent.

13.3.1 Exclusion of Women, Minorities, and Children

Women and minorities are not automatically excluded. Participants must meet all of the inclusion criteria listed in Section 5.2 and none of the exclusion criteria listed in Section 5.3 to be eligible to participate. Subjects aged <18 years will not be included in this study.

13.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The CRA or other authorized representatives of the sponsor may inspect all documents and records which require maintenance by the investigator, as described in Section 12.2.

13.5 Treatment Plan in the Event of Study Discontinuation

In the event the study is prematurely discontinued, arrangements will be made for the subjects' care to continue according to local standard clinical practice. Any subject still in the treatment period at the time of premature study discontinuation will have an early termination visit scheduled and the assessments listed in Section 7.5 performed. A final follow-up visit should be performed within 4 weeks following the early termination visit, as described in Section 7.4.

At the end of the study, subjects randomized to the placebo arm will not be crossed over to GKT137831 treatment.

13.6 Future Use of Stored Samples

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject.

Collected samples may be transferred for analysis to the Sponsor, or to other laboratories working for the Sponsor.

Biological samples will be stored for the time established by regulatory requirements or destroyed after the final clinical study report has been finalized if storage is not required. There might be a new request for these samples to be used for purposes related to the QA of the laboratory tests described in this protocol, in which case they will be used for this purpose. This may include the assessment of the quality of current tests, the maintenance or improvement of these tests, the development of new test methods for the markers described in this protocol, as well as making sure that new tests are comparable to previous methods and work reliably.

If study results suggest that further investigations using stored biological samples are warranted, these tests might be carried out on an exploratory basis. In addition, biological samples may be used by the Sponsor or their research partners for further research that is not related to the disease or the product under study. This testing will be done on anonymized samples (meaning that any identification linking the subject to the sample is destroyed). Subjects will be asked to sign an additional, separate consent form for this optional testing and refusal of consent will not affect their possibility of participating in the study.

13.7 Insurance

The Sponsor has established an insurance policy for the total anticipated duration of the study, covering the subjects with respect to the risks involved in taking part in this study in accordance with this protocol. In the case of injury or disability deriving from participation in the study, subjects are requested to inform the Investigator or his/her staff responsible for the study at the institution without delay.

14 DATA HANDLING AND RECORD KEEPING

The Investigator is responsible for ensuring the accuracy, completeness, legibility and timeliness of the source data. Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Investigator.

The Sponsor and/or its designee, Cmed, will provide guidance to investigators on recording trial data in the eCRF.

14.1 Data Capture Methods

Clinical data (including AEs, concomitant medications, safety assessments and outcome measures) will be entered by the site staff into a 21 CFR Part 11-compliant data capture system provided by Cmed. Clinical laboratory data will be entered by a central laboratory, with reports provided electronically to the sites for their records and to Cmed for loading into the database. The data capture system includes password protection and internal quality checks, such as automatic range checks, to identify data appearing inconsistent, incomplete or inaccurate.

CRO personnel will be responsible for training of investigator-designated site staff on the correct use of the EDC system. Authorized study staff will only be given access once they have received training.

Subjects will be provided with separate paper-based PBC-40 questionnaires and pruritus VAS at baseline/Day 1 and Weeks 12 and 24 (Visits 2, 5 and 7, respectively). Authorized site staff will then enter the responses provided in the eCRF with the original questionnaires being retained as part of the source documents.

14.2 Study Site Responsibilities

All data requested on the eCRF must be recorded. Data will be transcribed by authorized personnel at the study site from the source documents into the eCRF for enrolled subjects. All information on the eCRF must be traceable to these source documents. All electronic entries (including any changes or updates) will be traceable through the system. Only the Principal Investigator or authorized staff may enter or modify data in the database using their unique password and User Identification. The Investigator must certify that the data entered in the eCRFs are complete and accurate by electronically signing the eCRF.

14.3 Data Management Responsibilities

The Cmed Data and Analytics department will serve as the Statistical and Data Management Center for this study and will be responsible for data management, quality review, analysis and reporting of the study data.

Data management staff at the CRO will review the data in the eCRFs according to their internal SOPs and systematically validate the data using appropriate electronic checks in addition to relevant manual checks. For any errors identified in the data, the CRO will generate a formal query to be addressed by the investigational site staff within the EDC system.

For classification purposes, concomitant medications, AEs and medical history entered into the eCRF will be coded using relevant medication and medical term dictionaries. These will be specified in the study-specific Data Management Plan.

14.4 Study Records Retention

After the trial is completed, the investigator will receive a CD-ROM with the eCRFs of the subject data for the site for archiving at the investigational study site. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained.

14.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All major deviations must be promptly reported to Cmed and the Sponsor.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The Investigator and his/her staff are responsible for knowing and adhering to their IRB/IEC requirements.

15 PUBLICATION POLICY

The Sponsor shall retain the ownership of all data. When the study is complete the Sponsor shall arrange the analysis and tabulation of data. A clinical study report shall then be prepared, which may be used for publication, presentation at scientific meetings or submission to regulatory authorities. All proposed publications based on this study must be subject to the Sponsor's approval requirements.

Prior to subject enrollment the study will be registered with ClinicalTrials.gov. A summary of the study results will be published ClinicalTrials.gov.

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APPENDICES

APPENDIX A: SCHEDULE OF EVENTS

Study period	**Screening**	**Double-blind treatment period**						**Follow-up**
Study weeks	**-4 to -1**	**Baseline (Day 1)[1]**	**Week 2 ±3 days**	**Week 6 ±3 days**	**Week 12 ±3 days**	**Week 18 ±3 days**	**Week 24 ±3 days[9]**	**Week 28 ±3 days**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Informed consent	X							
Determination of eligibility[2]	X	X						
Demographics and medical history	X							
Body weight		X					X	
Height and oral or tympanic temperature	X							
Pregnancy test[3]	X	X	X	X	X	X	X	X
Blood sample for viral serology (HIV antibodies 1 and 2, hepatitis B surface antigen and hepatitis C virus antibodies)	X							
Optional collection of DNA sample		X						
Blood samples for the determination of anti-mitochondrial antibodies (anti-AMA, anti-GP210, anti-SP100, antibodies against the major M2 components [PDC-E2, 2-oxo-glutaric acid dehydrogenase complex])	X							
Blood samples for markers of inflammation (hsCRP, fibrinogen)[4]		X	X	X	X	X	X	X
Blood samples for optional markers of inflammation and liver injury (IL-6, CK-18)[4]		X			X		X	
Blood samples for markers of fibrosis (ELF score, collagen fragments)[4]		X			X		X	
Blood samples for optional assessments of immunological markers (IgM, IL-4, IL-12, IL-17A, IFN-γ)[4]		X			X		X	
Blood samples for total bile acids[4]		X			X		X	
Blood samples for optional assessments of bile acid metabolism (serum C4 and FGF-19)[4]		X			X		X	
Blood samples for optional additional biomarkers[4]		X			X		X	X
Blood samples for optional metabolomic studies[4]		X			X		X	
Transient elastography (FibroScan® or similar technology)		X					X	
Safety hematology and biochemistry including blood lipids, triglycerides, liver function tests and INR	X	X	X	X	X	X	X	X

Study period	Screening	Double-blind treatment period						Follow-up
Study weeks	**-4 to -1**	**Baseline (Day 1)[1]**	**Week 2 ±3 days**	**Week 6 ±3 days**	**Week 12 ±3 days**	**Week 18 ±3 days**	**Week 24 ±3 days[9]**	**Week 28 ±3 days**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Thyroid function tests		X	X	X	X	X	X	
Urinalysis (dipstick)	X				X		X	X
Subject questionnaires (PBC 40, Pruritus VAS)		X			X		X	
Pulse rate, SBP and DBP	X	X	X	X	X	X	X	X
12-lead ECG[5]	X		X		X		X	
Physical examination[6]	X	X	X	X	X	X	X	X
Prior and concomitant medication recording[7]	X	X	X	X	X	X	X	X
Randomization		X						
Dispense IMP[8]		X	X	X	X	X		
Supervise subject during first administration of IMP		X						
Blood samples for PK[9]			X		X	X		
Recording of AEs	X	X	X	X	X	X	X	X
Subject compliance with treatment			X	X	X	X	X	

Keys:

1. Day 1 (Visit 2) is also considered baseline of the 24-week double-blind treatment period (except for the determination of the primary endpoint where the mean of Screening (Visit 1) and Day 1 values will be defined as baseline).
2. Eligibility to enter the double-blind treatment period will be determined during Visits 1 and 2. Not every eligibility assessment needs to be repeated at Visit 2 i.e., confirmatory laboratory and diagnostic tests. Subjects with AEs at baseline may need to be withdrawn in accordance with the eligibility and withdrawal criteria.
3. At Screening (Visit 1), a serum pregnancy test will be performed. At all subsequent visits including baseline (Visit 2), urine pregnancy tests will be performed.
4. For the Baseline visit and all subsequent visits, subjects must remain fasted overnight from 10 pm and bring their morning dose to the clinic. Pre-dose samples will be collected and subjects will receive a breakfast meal. Subjects will then self–administer the morning dose up to 30 minutes after eating their meal. Post dose samples will be collected as described.
5. Post-dose, 12-lead ECG is to be performed around T_{max}, (i.e. 1 to 4 hours post dose).
6. A complete physical examination will be performed during the Screening visit (Visit 1) and at the follow-up (Visit 8). On all other visits a symptoms-directed physical examination will be performed.
7. Prior medication will be recorded at Screening only.

8. The IMP will be orally self-administered BID, once in the morning and once in the evening with meals or up to 30 minutes after eating a meal.
9. PK sampling: one pre-dose PK sample will be collected. Subjects must bring their morning dose to the clinic) and two post-dose samples (first sample to be taken 1-2 hours after morning dose; second sample to be taken before subject leaves clinic but no earlier than 2 hours after the first post-dose sample) at Weeks 2 and 12 (Visits 3 and 5). A single post-dose sample (to be taken before subject leaves clinic at 3-4 hours after morning dose) at Week 18 (Visit 6).
10. Week 24 (Visit 7) is the End of Treatment visit. Subjects who discontinue treatment before Week 24 will have an Early Termination Visit. The assessments to be performed at the Early Termination visit are the same as those at the Week 24 Visit, but must be recorded on the Early Termination eCFR page Subjects should return for a Final Study visit (Visit 8) within 4 weeks after the End of Treatment or Early Termination visit.

APPENDIX B: SCHEDULE OF BLOOD SAMPLING

	Blood Volume (mL)								Total Volume in Study
Assessment	**Screening**	**Double-blind treatment period**						**Follow-up**	
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
Serum pregnancy test (if applicable)	2	-	-	-	-	-	-	-	2
HIV1, HIV2, HBVAb, HCVAb	4	-	-	-	-	-	-	-	4
DNA sample	-	5	-	-	-	-	-	-	5
Antibodies: anti-AMA, anti-GP210, anti-SP100, anti-M2 component	1	-	-	-	-	-	-	-	1
PD inflammation markers (hsCRP, fibrinogen)	-	4	4	4	4	4	4	4	28
Optional PD inflammation markers (IL-6, CK-18)	-	4	-	-	4	-	4	-	12
PD fibrosis markers (ELF score, collagen fragments)	-	4	-	-	4	-	4	-	12
Optional immunological markers (IgM, IL-4, Il-2, IL-17A, IFNγ)	-	3	-	-	3	-	3	-	9
Mandatory total bile acids	-	2	-	-	2	-	2	-	6
Optional bile acid metabolism markers (serum C4, FGF-19)		2			2		2		6
Optional additional biomarkers	-	3	-	-	3	-	3	3	12
Optional PD metabolomic markers	-	1.5	-	-	1.5	-	1.5	-	4.5
Chemistry panel	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	68
Hematology panel	3	3	3	3	3	3	3	3	24
INR	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	21.6
Thyroid panel	-	2	2	2	2	2	2	-	12
PK	-	-	6	-	6	2	-	-	14
Total volume to be drawn at each visit / in the study	21.2	44.7	26.2	20.2	45.7	22.2	39.7	21.2	241.1

APPENDIX C: DECLARATION OF HELSINKI 2013

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by

individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and

standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

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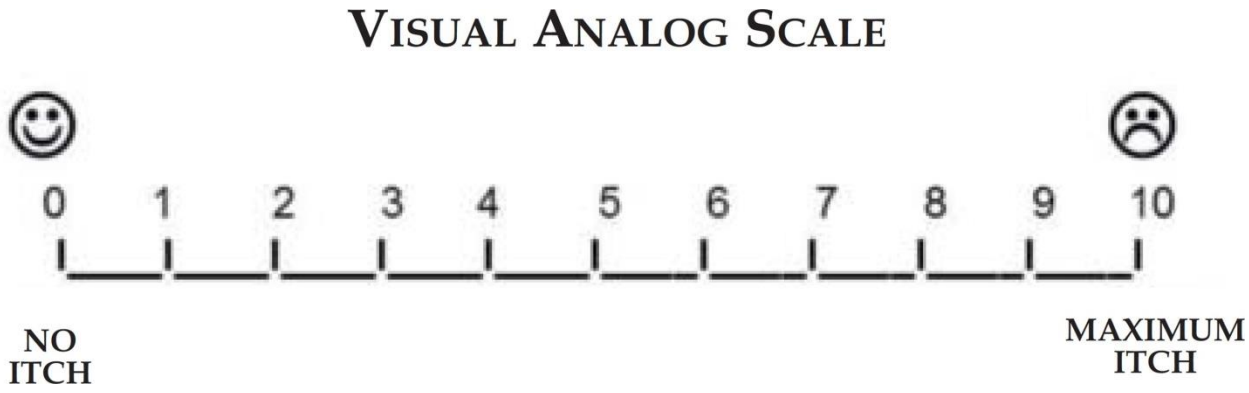
APPENDIX D: PRURITUS VAS

The VAS is composed of a 10-cm line divided into a scale from 0 to 10, and subjects should indicate the score that best represents the intensity of their itching (0 = no itch, 100 = severe, continuous, day and night intolerable itch)



APPENDIX E: PBC-40 QUESTIONNAIRE

Patient ID	Date:

For each statement, please circle the response that comes closest to how you feel. If any of the statements do not apply to you please circle 'does not apply'.

Can you say how often the following statements about digestion and diet applied to you IN THE LAST FOUR WEEKS?

1	I was able to eat what I liked	Never	Rarely	Sometimes	Most of the time	Always	
2	I ate or drank only a small amount, and still felt bloated	Never	Rarely	Sometimes	Most of the time	Always	
3	I felt unwell when I drank alcohol	Never	Rarely	Sometimes	Most of the time	Always	Did not apply /never drink alcohol

And IN THE LAST FOUR WEEKS, how often did you experience any of the following?

4	I had discomfort in my right side	Never	Rarely	Sometimes	Most of the time	Always
5	I had dry eyes	Never	Rarely	Sometimes	Most of the time	Always
6	My mouth was very dry	Never	Rarely	Sometimes	Most of the time	Always
7	I had aches in the long bones of my arms and legs	Never	Rarely	Sometimes	Most of the time	Always

Some people with PBC experience itching. How often did you experience itching IN THE LAST FOUR WEEKS? If you did not itch, please circle 'does not apply'

8	Itching disturbed my sleep	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/ no itch
9	I scratched so much I made my skin raw	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/no itch
10	I felt embarrassed because of the itching	Never	Rarely	Sometimes	Most of the time	Always	Did not apply/no itch

Fatigue can also be a problem for many people with PBC. How often did the following statements apply to you IN THE LAST FOUR WEEKS?

11	I had to force myself to get out of bed	Never	Rarely	Sometimes	Most of the time	Always
12	I had to have a sleep during the day	Never	Rarely	Sometimes	Most of the time	Always
13	Fatigue interfered with my daily routine	Never	Rarely	Sometimes	Most of the time	Always
14	I felt worn out	Never	Rarely	Sometimes	Most of the time	Always
15	I felt so tired, I had to force myself to do the things I needed to do	Never	Rarely	Sometimes	Most of the time	Always
16	I felt so tired, I had to go to bed early	Never	Rarely	Sometimes	Most of the time	Always
17	Fatigue just suddenly hit me	Never	Rarely	Sometimes	Most of the time	Always
18	PBC drained every ounce of energy out of me	Never	Rarely	Sometimes	Most of the time	Always

The next section is about the effort and planning that can be involved in living with PBC. Thinking about THE LAST FOUR WEEKS, how often did the following statements apply to you?

19	Some days it took me a long time to do anything	Never	Rarely	Sometimes	Most of the time	Always
20	If I was busy one day I needed at least another day to recover	Never	Rarely	Sometimes	Most of the time	Always
21	I had to pace myself for day-to-day things	Never	Rarely	Sometimes	Most of the time	Always

The following statements are about the effects that PBC may have on things like memory and concentration. Thinking about THE LAST FOUR WEEKS, how often did the following statements apply to you?

22	Because of PBC I had to make a lot of effort to remember things	Never	Rarely	Sometimes	Most of the time	Always
23	Because of PBC I had difficulty remembering things from one day to the next	Never	Rarely	Sometimes	Most of the time	Always
24	My concentration span was short because of PBC	Never	Rarely	Sometimes	Most of the time	Always

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25	Because of PBC, I had difficulty keeping up with conversations	Never	Rarely	Sometimes	Most of the time	Always
26	Because of PBC, I found it difficult to concentrate on anything	Never	Rarely	Sometimes	Most of the time	Always
27	Because of PBC, I found it difficult to remember what I wanted to do	Never	Rarely	Sometimes	Most of the time	Always

Now some more general statements about how PBC may be affecting you as a person. How much do the following statements apply to you?

28	Because of PBC, I get more stressed about things than I used to	Not at all	A little	Somewhat	Quite a bit	Very much	
29	My sex life has been affected because of PBC	Not at all	A little	Somewhat	Quite a bit	Very much	Does not apply
30	Having PBC gets me down	Not at all	A little	Somewhat	Quite a bit	Very much	
31	I feel I neglect my family because of having PBC	Not at all	A little	Somewhat	Quite a bit	Very much	Does not apply
32	I feel guilty that I can't do what I used to do because of having PBC	Not at all	A little	Somewhat	Quite a bit	Very much	
33	I worry about how my PBC will be in the future	Not at all	A little	Somewhat	Quite a bit	Very much	

These statements relate to the possible effects of PBC on your social life. Thinking of your own situation, how much do you agree or disagree with them?

34	I sometimes feel frustrated that I can't go out and enjoy myself	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
35	I tend to keep the fact that I have PBC to myself	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
36	I can't plan holidays because of having PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
37	My social life has almost stopped	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree

The next section is about the impact that PBC may be having on your life overall. How much do you agree or disagree with the following statements?

38	Everything in my life is affected by PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
39	PBC has reduced the quality of my life	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
40	I can still lead a normal life, despite having PBC	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree

The next few questions are about your general health and well being:

A	In general, would you say your health is:	Excellent	Very good	Good	Fair	Poor
B	And how would you have rated it before you had PBC?	Excellent	Very good	Good	Fair	Poor
C	COMPARED TO ONE YEAR AGO, how would you rate your health in general now?	Much better	Somewhat better	About the same	Somewhat worse	Much worse

THANK YOU FOR TAKING THE TIME TO COMPLETE

APPENDIX F: CLOSE OBSERVATION PROCEDURES

1. Interrupt the investigational agent administration
2. Repeat liver biochemistries and additional testing within 24-72 hours
3. Monitor patient twice or thrice a week until liver biochemistries (ALT, AST, alkaline phosphatase, total bilirubin, and coagulation profile [INR]) resolve, stabilize or return to within baseline values
4. Monitor liver biochemistries once a week if abnormalities stabilize and the patient is asymptomatic
5. Obtain a detail history for symptoms assessment: appearance or worsening of clinical symptoms of hepatitis (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia). If a patient is symptomatic, the drug must be discontinued immediately and a potential DILI evaluation must be performed.
6. Obtain a more detailed history of symptoms and prior or concomitant diseases;
7. Obtain a history for concomitant medications, acetaminophen, dietary supplements, herbal remedies, other over the counter medications, recreational drug use, and special diets
8. If possible quantify the alcohol consumption to assess for alcoholic hepatitis
9. Obtain a history of exposure to environmental chemical agents.
10. If INR is also elevated, a trial of intravenous vitamin K administration may be considered, especially in cholestatic patients.

Follow-Up Procedures for patient(s) who meet *potential DILI evaluation criteria*:

1. Viral hepatitis serology including:
    a. Hepatitis A IgM antibody;
    b. Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM);
    c. Hepatitis C RNA;
    d. Hepatitis E IgM antibody.
    e. Cytomegalovirus IgM antibody;
    f. Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); however IgM antibodies must be sent out asap
    g. Blood sample for pharmacokinetic (PK) analysis, obtained within 12 hours of last dose. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the participant's best approximation.
    h. Serum Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
    i. Fractionate bilirubin, if total bilirubin >2xULN
    j. Assess for peripheral eosinophilia
    k. Assess for hypoxic/ischemic hepatopathy; and biliary tract disease

The following are required for patients who meet the stopping criteria for both ALT and total bilirubin OR experiences clinical symptoms of hepatitis:

1. Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies and quantitative total immunoglobulin G (IgG or gamma globulins).
2. If required evaluation of competing undiagnosed liver disease (hemochromatosis, Wilson's disease, alpha-1 anti-trypsin deficiency)

3. Serum acetaminophen levels OR serum acetaminophen adducts by HPLC assay (quantifies potential acetaminophen contribution to liver injury in participants with definite or likely acetaminophen use in the preceding week).
4. Liver imaging (ultrasound, magnetic resonance, or computerized tomography) to evaluate liver disease.
5. A Liver Biopsy

Treatment with investigational agent could be re-initiated if these abnormalities stabilize, return to pre-trial baseline or normalize and a causative agent was found.

Investigational agent must be discontinued and patient must be followed until the clinical and laboratory abnormalities stabilize or normalize if the following criteria are met:
1. If close monitoring of a patient is not possible.
2. In presence of total bilirubin elevation (>2 x ULN or >1.5 x baseline); with any degree of aminotransferase elevation; AND if there is appearance of symptoms i.e., fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).
3. If any degree of total bilirubin, ALT, or AST elevation recurs following re-challenge with study drug.