PROTOCOL: SHP626-201

TITLE:	A Phase 2 Double-blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)
DRUG:	Volixibat potassium (SHP626)
IND:	123,847
EUDRACT NO.:	2016-000203-82
SPONSOR:	Shire Human Genetic Therapies, Inc. 300 Shire Way Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Philip Newsome MD Director of Centre for Liver Research University of Birmingham Edgbaston, Birmingham B15 2TT UK
NATIONAL/ COORDINATING INVESTIGATOR/UK	Guruprasad P Aithal MBBS, MD, FRCP, PhD Head of the Division, Nottingham Digestive Diseases Centre Director, NIHR Nottingham Digestive Diseases Biomedical Research Unit Nottingham University Hospitals and University of Nottingham Queen's Medical Centre Nottingham NG7 2UH
PROTOCOL HISTORY:	Amendment 4: 25 August 2017 Amendment 3: 22 March 2017 Amendment 2: 19 July 2016 Amendment 1: 8 April 2016 Original Protocol: 24 February 2016

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature:	**Date:** 25 Aug 2017
Melissa Palmer, MD, FAASLD Global Clinical Development Lead (GCDL) - Hepatology	

Investigator's Acknowledgement

I have read this protocol for Shire Study SHP626-201.

Title: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS VERSION

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **4**	**Amendment Date** **25 August 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Emergency Contact Information	Updated information for the Shire study physician and ICON medical monitor.	• To provide updated and correct information.
Synopsis	Reduced planned study period from July 2020 to January 2020.	• Reduced planned study period due to elimination of the previously planned pause in enrollment.
Synopsis	Extended maximum duration of screening period from 56 days to 70 days.	• To accommodate additional tissue for liver biopsy central reads in addition to other procedures.
Synopsis, Section 9.6	The "Number of Subjects" was updated to reflect new numbers following elimination of the previously planned enrollment pause.	• To provide updated and correct information.
Synopsis	The "Methodology" section was updated to reflect new numbers following elimination of the previously planned enrollment pause.	• To provide updated and correct information.
Synopsis; Section 4.2	Updated exclusion criterion #15 to indicate that subjects with TB >2 ULN at screening would be excluded.	• To provide correct information consistent with the rest of the protocol.
Table 1, Section 3.1, Section 7.1, Section 7.1.1	Lengthened screening window from 8 weeks (56 days) to 10 weeks (70 days). Updated study design flow chart to reflect extended screening window.	• To accommodate additional tissue for liver biopsy central reads in addition to other delays.
Section 3.1 , Section 9.6	Updated text to provide revised numbers of subjects expected to be enrolled, following elimination of the previously planned enrollment pause.	• To provide updated and correct information.
Section 3.1	Revised the capping number for F0 subjects; this will be capped at 88 if 1 dose is dropped after the interim analysis and at 78 if 2 doses are dropped (% remains the same at 30%) following elimination of the previously planned enrollment pause.	• To provide updated and correct information.

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **4**	**Amendment Date** **25 August 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Section 3.1, Section 9.5.1	Removed sentence: "Study enrollment will be paused after 92 subjects have been randomized."	• This reflects elimination of the previously planned pause in enrollment.
Section 3.2	Revised subject's maximum duration of participation as expected to be approximately 434 days.	• This was revised as the screening period was lengthened to 10 weeks and this duration also did not previously include the screening period.
Section 5.2.1	Removed the phrase: "then the Shire GCDL".	• To provide updated and correct information.
Section 6.2.2	Revised the capping number for F0 subjects; this will be capped at 88 if 1 dose is dropped after the interim analysis and at 78 if 2 doses are dropped (% remains the same at 30%) following elimination of the previously planned enrollment pause.	• To provide updated and correct information.
Section 6.2.3 , Section 7.1.3	Removed sentence: "All assessments should be completed at least 30 minutes prior to administration of the IP."	• This stipulation is not necessary for this study as determined by Phase 1 final results.
Section 6.2.4, Section 8.2.2, Section 8.2.3	Replaced "Shire GCDL" with "Shire study physician"	• To provide correct information/ guidance.
Section 7.1, Section 7.1.1	Replaced "medical monitor" with "Shire study physician"	• To provide correct information/ guidance.
Section 9.5.1	Removed the sentence: "Subjects will be randomized to 1 or 2 volixibat doses or to PBO."	• The sentence was redundant and this idea is presented elsewhere in the same paragraph.
Section 9.5.1	Added text indicating that the IA will also be performed by a gastroenterologist and hepatologist.	• To provide correct information/ guidance.
Section 10	Added text indicating that compliance with current applicable regulations also constitutes compliance with the ethical principles in the Declaration of Helsinki.	• To provide correct information/ guidance.

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **4**	**Amendment Date** **25 August 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Section 10.1.1, Section 10.2.1	Added reference to ICH GCP E6 R2 2017 guidelines.	• To provide correct information/ guidance.
Appendix 5	Updated formatting to align with source document, and eliminated irrelevant footnote to this table. Added definition of baseline.	• To improve clarity of the information in the table and adhere to the source table.
Throughout protocol	Minor changes to wording.	• To improve clarity

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious Adverse Events (AEs) Required by the Protocol Form within 24 hours to:

Shire Global Drug Safety Department

Preferred method: scan and e-mail to globalpharmacovigilance@shire.com
OR fax to +44(0) 1256 89 4715 (Global); or +1 866 557 4473 (North America)

AND

Notification and/or a copy of this form must also be sent to the Shire study physician by e-mail using the details below:

Melissa Palmer, MD, FAASLD, GCDL-Hepatology

e-mail: mpalmer@shire.com

For protocol- or safety-related issues during normal business hours 9:00 a.m. to 5:00 p.m. (Eastern Time), the investigator must contact the Contract Research Organization (CRO) Medical Monitor:

ICON Medical Monitor: Jack Martin, MD
Telephone number: +1 215 583 2257
Fax number: +1 215 616 3096
Mobile number: +1 215 593 9095
E-mail address: Jack.Martin@iconplc.com

For protocol- or safety-related issues outside of normal business hours, the investigator must contact ICON's 24/7 Medical Emergency Coverage:

Chargeable global telephone number: +1 919 674 5468

NOTE: Investigative sites will be provided country-specific toll-free telephone numbers. Please refer to this document, as applicable. Countries without a toll-free number will need to dial the chargeable number noted above.

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

Please use the information below as applicable to report the Product Quality Complaint:

Origin of Product Quality Complaint	**E-mail Address**
North and South America	PQC@shire.com
European Union and Rest of World	PQCROW@shire.com

Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
+1-888-300-6414 or +1-800-828-2088

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ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPPT	activated partial thromboplastin time
ASBT	apical sodium-dependent bile acid transporter
ASBTi	ASBT inhibitor
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BA	bile acid
BMI	body mass index
C4	7-alpha-hydroxy-4-cholesten-3-one
CRA	clinical research associate
CRC	clinical research center
CRO	contract research organization
DMC	data monitoring committee
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimension-5 Level Questionnaire
ECG	electrocardiogram
EOS	end of study
EU	European Union
FAS	full analysis set
FBA	fecal bile acid
FDA	Food and Drug Administration
FXR	farnesoid X receptor
GCDL	Global Clinical Development Lead
GCP	Good Clinical Practice
GDS	Global Drug Safety
GGT	gamma glutamyl transferase
GLP-1 RA	glucagon-like peptide-1 receptor agonists
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus

HBVDNA	hepatitis B virus deoxyribonucleic acid
HCV	hepatitis C virus
HCVAb	hepatitis C virus antibody
HCVRNA	hepatitis C virus ribonucleic acid
HDL-C	high-density lipoprotein-cholesterol
HFD	high fat diet
HFF	hepatic fat fraction
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HOMA-%B	homeostasis model assessment in β-cell function
HOMA-IR	homeostasis model assessment insulin resistance
HRQoL	health-related quality of life
IA	interim analysis
IAS	interim analysis set
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	investigational new drug
INR	international normalized ratio
IP	investigational product
IR	insulin resistance
IRB	Institutional Review Board
IRT	interactive response technology
LDL-C	low-density lipoprotein-cholesterol
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MOA	mechanism of action
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NOAEL	no observed adverse effect level
PBC	primary biliary cirrhosis

PBO	placebo
PDFF	proton density fat-fraction
PO	by mouth
POC	proof of concept
PRO	patient-reported outcome
PSC	primary sclerosing cholangitis
PT	prothrombin time
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAF	steatosis, activity, and fibrosis score
SAP	statistical analysis plan
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TA	therapeutic area
TB	total bilirubin
TEAE	treatment-emergent adverse event
TGR5	transmembrane G protein-coupled receptor
TPN	total parenteral nutrition
TSH	thyroid-stimulating hormone
TZD	thiazolidinediones
ULN	upper limit of normal
USA	United States of America
VAS	visual analogue scale

STUDY SYNOPSIS

Protocol number: SHP626-201	**Drug:** Volixibat potassium (SHP626)
Title of the study: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)	
Number of subjects (total and for each treatment arm): The number of subjects screened and randomized will be dependent upon the number of dosing arms dropped following the interim analysis (IA). • Approximately 899 subjects will be screened to randomize 292 subjects (81 per continued dosing arm, approximately 49 per dropped dosing arm) to achieve 201 completers if three arms (2 volixibat and 1 placebo [PBO]) continue after the interim analysis (one dose is dropped after the IA). • Approximately 807 subjects will be screened to randomize 260 subjects (81 per continued dosing arm, approximately 49 per dropped dosing arm) to achieve 134 completers if two arms (1 volixibat and 1 PBO) continue after the IA (two doses are dropped after the IA).	
Investigator(s): Multicenter study	
Site(s) and Region(s): Anticipated regions: US, Canada, and EU Estimated number of sites planned: 60 to 80	
Study period (planned): July 2016 to January 2020	**Clinical phase:** 2
Objectives: **Primary:** To evaluate the effect of volixibat compared to PBO on liver histology **Secondary:** • To evaluate the safety and tolerability of volixibat compared to PBO • To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI) • To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual nonalcoholic fatty liver disease (NAFLD) activity score (NAS) components and fibrosis stage) • To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis) • To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry • To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c]) • To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)	
Rationale: Currently, there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its mechanism of action (MOA) and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.	
Investigational product, dose, and mode of administration: • Volixibat 5, 10, and 20 mg and matched PBO capsules by mouth (PO) once daily (QD). Investigational product (IP) should be given 30 minutes prior to the first meal of the day containing approximately 10-	

20 grams of fat. Also see Section 6.2.3 (Dosing).

- Identical PBO will be used as comparator
    - Subjects should take the IP at the same time each day and should not take more than one dose in a day if they miss a dose. If a dose is missed at the normally scheduled time, the subject can make up the dose that day as long as no more than 1 dose is taken in a 10-hour period.

Methodology:

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have received 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg) once daily (QD) or PBO in a 1:1:1:1 ratio. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 88 if 1 dose is dropped after the IA and at 78 if 2 doses are dropped (approximately 30% of the total number of subjects). Depending on the outcome of the IA, one or more treatment arms will be discontinued or the study may be terminated. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

Inclusion and exclusion criteria:

Inclusion Criteria:

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally-authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and 30 days following the last dose of the IP.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F0-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

Exclusion Criteria:

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV [defined as HCVAb positive] without a current or prior history of a detectable HCVRNA) will be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the IP (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve

is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.

4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver biopsy and/or MRI within 6 months of screening, but experienced a weight change ≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.
5. Contraindications to MRI (ie, claustrophobia, coronary stents, coronary implantable devices, girth, etc.). Stents or other devices may be allowed, at the investigator's discretion, if they do not interfere with the functioning of the MRI machine.
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation.
8. Uncontrolled diabetes defined as HbA1c of ≥9.5% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening, or a sliding scale of insulin is allowed as long as the subject's HbA1c remains < 9.5%.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to qualifying liver biopsy, or initiated any time after qualifying liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST >7 times upper limit of normal (ULN) at screening.
12. Serum ALT >7 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3.
15. Total bilirubin (TB) >2 times ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$L
17. Medical history of impaired hemostasis or current use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie, 81mg, aspirin (ASA) or clopidogrel (Plavix), will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP

- Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study

23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.
25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior sponsor approval).
31. Subjects who are employees at the unit of the investigational site that is conducting the study.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 70 days
- Planned duration of treatment period: 336 days
- Planned duration of follow-up: 28 days

Endpoints and statistical analysis:

Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an adverse event (AE) or reporting the absence of AEs).

Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the time of the data cut for the IA.

Screened Set will consist of all subjects who have signed an informed consent.

Randomized Set will consist of all subjects in the Screened Set who have been randomized into the study.

Efficacy Endpoints:

- ***Primary:*** Binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS.
- ***Secondary:***
  - Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
  - Change from baseline to Week 48 on hepatic steatosis as measured by MRI-PDFF.
  - Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH Clinical Research Network (CRN))
  - Resolution of NASH (defined as total absence of ballooning [score = 0] absent or mild

inflammation [score 0-1], steatosis can be present [score 0-3]), without worsening of fibrosis as assessed by liver histology at week 48.

- Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
  - alanine aminotransferase (ALT)
  - aspartate aminotransferase (AST)
  - alkaline phosphatase (ALP)
  - gamma glutamyl transferase (GGT)
  - total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
  - fasting serum glucose levels
  - insulin levels
  - HbA1c
- Change from baseline to Week 48 on serum lipids measured by:
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

Primary hypotheses:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.
- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

The FAS will be used to assess the primary efficacy endpoint. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. Subjects with missing data for the primary efficacy endpoint at the Week 48 visit will be considered as non-responders. The test will be stratified by presence or absence of Type 2 diabetes mellitus (T2DM) at baseline, and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level. Histology will be read by one central hepatohistopathologist who will use the NASH CRN standard scoring system – NAS. The SAF Steatosis (S), Activity (A), and Fibrosis (F) scoring system will be determined for exploratory purposes.

The Safety Analysis Set will be used to assess the safety endpoints including AEs (including changes from baseline in physical examination findings), vital signs, ECGs, and clinical laboratory tests (chemistry, hematology, coagulation and urinalysis).

AEs will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as AEs that started or worsened on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be presented by system organ class, preferred term, and by treatment group. TEAEs will be further summarized by severity and the relationship to the IP. AEs related to the IP, AEs leading to withdrawal, SAEs, and death will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

For safety parameters, baseline is defined as the last assessment prior to the first dose of the IP.

Planned Interim Analysis:

An IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat

each compared to PBO.

Tolerability will be assessed by the number of discontinuations due to any one TEAE, presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Efficacy at the IA will be based on reduction of steatosis or ALT. Steatosis is assessed by MRI-PDFF. Depending on the results from the IA, one or more doses of volixibat may be discontinued or the study may be terminated. If the study is not terminated, subjects will receive a total of 48 weeks of the IP.

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9[c] EOS**	**10 In-Clinic**
Week	**-10 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-70 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Informed consent[d]	X									
Inclusion/exclusion criteria	X	X (review)								
Demography, medical & medication history	X									
Physical examination	X	X					X		X	X
Height[e], weight[f], waist circumference and waist:hip ratio	X	X	X	X	X	X	X	X	X	X
Vital signs[g]	X	X	X	X	X	X	X	X	X	X
PRO (EQ-5D-5L)		X					X		X	
Liver biopsy[h]	X								X	
MRI[i]	X						X[j]		X	
ECG (12-lead)	X	X				X			X	
Biochemistry and Hematology[k]	X	X	X	X	X	X	X	X	X	X
Serum Glucose[l]	X	X	X	X	X	X	X	X	X	X
Urinalysis[m]	X	X							X	
Urine Drug and Blood Alcohol Tests	X	X								
Urine Pregnancy Test[n]	X	X	X	X	X	X	X	X	X	X
Lipid Panel[0]	X	X	X	X	X	X	X	X	X	X
Coagulation Panel[p]	X	X		X			X		X	

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9[c] EOS**	**10 In-Clinic**
Week	**-10 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-70 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Vitamins A, D, and E[t]		X		X			X		X	
HbA1c[k]	X	X	X	X	X	X	X	X	X	X
Serum Liver-Related Blood Tests[k, q]	X	X	X	X	X	X	X	X	X	X
Insulin[k]	X	X		X		X	X	X	X	X
HIV, Hepatitis B/C[k, r]	X									
Thyroid testing[k, s]	X	X		X		X	X		X	X
C4 Sampling		X				X	X		X	X
IRT Accessed	X	X	X	X	X	X	X	X	X	X
Randomization		X								
IP Dispensed[t]		X	X	X	X	X	X	X		
IP Returned/Accountability & Compliance Assessed			X	X	X	X	X	X	X	
Stool Assessment[u]		X	X			X		X	X	X
Adverse Events[v]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X

ECG=electrocardiogram; EOS=end of study; HOMA=Homeostasis Model Assessment; IA=interim analysis; IP=investigational product; IRT=Interactive Response Technology; MRI=magnetic resonance imaging; PRO=patient-reported outcome.

[a] Visit Windows (calculated from Visit 2): Bi-weekly (Visits 3-4): +/- 3 days; Monthly (Visits 5-6, Visit 10): +/-5 days; Tri-monthly (Visits 7-9/DC): +/- 7 days.

[b] Subjects will be reminded not to eat prior to their scheduled visit. Additionally, during the double-blind treatment period, they should not take their study drug prior to the visit. They should bring their study drug with them to the visit to take 30 minutes prior to their first meal of the day containing approximately 10-20 grams of fat. Also see Section 6.2.3

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9[c] EOS	10 In-Clinic
Week	-10 to 0	0	2	4	8	12	24	36	48	52
Study Day	-70 to -1	0	14	28	56	84	168	252	336	364

[c] Subjects are to complete the Visit 9 liver biopsy and MRI procedures within +14 days of Visit 9. All other Visit 9 assessments are to be completed within a window of +/- 7 days.

[d] Subjects who completed screening and were designated as a screen failure may be rescreened. However, rescreened subjects must begin the screening procedure again, must be re-consented and will be assigned a new subject number.

[e] Height to be measured at screening only.

[f] BMI to be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7 and 9/DC.

[g] Vital signs to include oral, temporal, or tympanic temperature, sitting blood pressure, pulse, and respiratory rate.

[h] Biopsy performed within 6 months of screening can be used. All biopsies will be centrally read by a hepatic histopathologist. See Sections 7.1.1 and 7.1.3 for additional details.

[i] MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.

[j] MRI at Visit 7 (Week 24) for the Interim Analysis Set only.

[k] All blood tests are fasting blood tests. Subjects are not required to have Vitamin A, D, and E testing at screening (Visit 1).

[l] HOMA-IR and HOMA-%B: will be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7, 9/DC, and 10.

[m] Urinalysis to include oxalate testing.

[n] For all females of child-bearing potential (FOCP). Positive on-site urine dipstick results must have serum β-HCG testing performed by central lab.
Additional testing can be performed at the investigator's discretion.

[o] Lipid Panel includes fasting total cholesterol, HDL-C, LDL-C, and triglycerides.

[p] Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points to assess vitamin K level.

[q] Serum Liver-Related Blood Tests include ALT, AST, ALP, GGT, and total bilirubin.

[r] Hepatitis B/C testing includes HBcAb, HBsAg, HBVDNA and HCVAb (if HCVAb is positive an HCVRNA will be performed), respectively.

[s] Thyroid testing includes thyroid stimulating hormone (TSH) and triiodothyronine (T3).

[t] Investigational product may be dispensed at an unscheduled visit outside of this schedule as needed to replace lost or damaged product.

[u] Subjects will be queried about the number of stool evacuations during the 24- hour period before the clinical research center (CRC) visit and asked to describe the consistency of the softest stool during that 24- hour period using the Bristol Stool Chart.

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9[c] EOS	10 In-Clinic
Week	-10 to 0	0	2	4	8	12	24	36	48	52
Study Day	-70 to -1	0	14	28	56	84	168	252	336	364

[v] Adverse events will be collected beginning from the signing of informed consent. All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

1 BACKGROUND INFORMATION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide (Vernon et al., 2011), and is estimated to occur in 30-40% of adults in the United States and up to 30% of European adults. These numbers approach 95% in those with morbid obesity (Mathus-Vliegen et al., 2012). NAFLD ranges from simple steatosis, which is typically nonprogressive, to nonalcoholic steatohepatitis (NASH), which has a 20% likelihood of progression to advanced disease including fibrosis, cirrhosis and its complications including liver failure and the need for a liver transplant (Angulo, 2002). Hepatocellular carcinoma is also a complication of NASH that may occur with or without the presence of cirrhosis (Torres et al., 2012). NAFLD is typically associated with type 2 diabetes mellitus (T2DM) (Adibi et al., 2007; Loomba et al., 2012), central or visceral obesity (Souza et al., 2012), dyslipidemia (Assy et al., 2000; National Cholesterol Education Program, 2002), and hypertension (Donati et al., 2004). Together these conditions comprise the metabolic syndrome, and NASH is considered to be the hepatic component of this syndrome (Hamaguchi et al., 2005; Neuschwander-Tetri, 2005; Marchesini et al., 2005). Notably, NAFLD is a clinical condition occurring in individuals who do not drink excessive alcohol (>20 grams/day), yet have hepatic histology which is indistinguishable from that seen with alcoholic excess. The pathophysiology of NASH is likely multifactorial and may include combinations of metabolic, genetic, environmental, and gut microbial factors.

Most individuals with NASH are asymptomatic or have nonspecific symptoms such as fatigue. They typically first come to medical attention incidentally following routine blood testing or on imaging studies performed routinely or during the evaluation of an unrelated condition. While ultrasound and magnetic resonance imaging (MRI) can detect the presence of steatosis (Reeder et al., 2011), a liver biopsy is required to diagnose NASH and the extent of liver fibrosis.

1.1 Indication and Current Treatment Options

There are currently no drugs approved for the treatment of NASH and it is estimated that there are between 6-16 million people in the United States with NASH, of which 600,000 have severe disease (Williams et al., 2011 and Torres and Harrison, 2008), with similar percentages reported throughout most areas of the world (World Gastroenterology Organisation, 2012). Treatment of associated metabolic comorbidities, weight reduction, and incorporation of an exercise routine remain the cornerstone of management. However, lifestyle changes are seldom successful. Thus, NASH represents a disease with an unmet medical need that is growing at an epidemic rate, and that if untreated, carries a risk of significant morbidity and mortality.

1.2 Product Background and Clinical Information

Volixibat potassium (SHP626; formerly LUM002), hereafter referred to as volixibat, is a highly selective inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that is being evaluated for the treatment of NASH.

Bile acids (BA) promote bile flow, activation of digestive enzymes, and micellization of fats and fat-soluble vitamins, thereby permitting their intestinal absorption. BAs serve as signaling molecules acting via receptors, such as farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR5), in the intestine, liver and other tissues which play an important role in regulating insulin homeostasis. (Halilbasic et al., 2013). Lipid peroxidation and oxidant stress have been proposed as one link between the accumulation of fat and subsequent injury (Day and James, 1998). All of these metabolic actions have important effects to prevent the ongoing liver damage in NASH.

High fat diet (HFD) fed mice treated with an ASBT inhibitor (ASBTi) (SC-435 a surrogate SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved insulin resistance (IR), and decreased NAS, predominantly steatosis. In addition the BA pool reflected increases in BA that were agonists to FXR and decreases in levels that were antagonist to the FXR (Rao et al., 2016). Miethke and colleagues found that pharmacological inhibition of the ileal, ASBT (SC-435 the surrogate molecule of volixibat), blocked progression of sclerosing cholangitis in mdr2–/– mice (Miethke et al., 2016). Beneficial effects in liver histology in these mice included a reduction in the severity of hepatic fibrosis, a decrease which correlated with a reduction of hepatic profibrogenic gene expression. While very encouraging, whether or not the findings in this mouse sclerosing cholangitis model can translate to histologic improvements in patients with NASH is unknown.

Volixibat, as a potent inhibitor of ASBT, increases BA excretion and facilitates signaling in the intestine that regulates serum and hepatic BA concentrations, glucose metabolism, serum cholesterol and fatty acid metabolism in the liver. The combined events resulting from inhibiting BA reuptake are hypothesized to have a positive metabolic, anti-inflammatory, anti-steatotic, and potentially antifibrotic effect that will lead to a therapeutic benefit for patients with NASH.

One of the factors contributing to the pathogenesis of NASH is abnormal cholesterol metabolism and the accumulation of free cholesterol in the liver. The free cholesterol is directly toxic to hepatocytes, which leads to inflammation and fibrosis (Musso et al., 2013). One treatment approach is to remove the cholesterol from the liver to decrease and possibly reverse the damage to the hepatocytes. This is one of the mechanisms by which SHP626 will treat NASH.

Due to the mechanism of action (MOA), volixibat is under development for the treatment of NASH and may also be able to improve the metabolic syndrome that is associated with NASH. Volixibat inhibits ASBT therefore BAs are excreted in the feces and this loss forces the liver to synthesize new BA which utilizes cholesterol in the liver and serum. Volixibat also has the potential to reduce IR, which is considered to be the most common underlying risk factor for the development of NASH (Pagano et al., 2002; Sanyal et al., 2001).

Volixibat was initially being evaluated for its use as an intervention for dyslipidemia. As a result of initial observations in animals that serum low-density lipoprotein cholesterol (LDL-C) and total liver cholesterol content could be decreased after administration of volixibat, additional work was undertaken to support the safety and tolerability of the drug in healthy volunteers. Clinical and non-clinical studies have demonstrated very low systemic exposure across species.

Oral administration of volixibat at doses up to 300 mg once daily and 50 mg administered daily for 14 days to healthy male subjects was generally safe. A Phase 1 study revealed that volixibat is tolerated at the 10 mg dose for 28 days and that there were trends towards increasing high-density lipoprotein cholesterol (HDL-C), decreasing LDL-C, and decreasing fasting glucose in patients with T2DM (LUM002-101 trial).

A healthy volunteer study investigating 12 days of varying levels of repeat doses of volixibat has provided key pharmacodynamic information for dose selection as measured by the effect of volixibat on excreted fecal bile acid (FBA) levels (SHP626-101). In addition, the study investigated safety and tolerability over the dose range that was considered for this Phase 2 study.

Data from Phase 1 studies support the position that volixibat is basically a non-absorbed drug that works locally in the GI tract and results in virtually no systemic exposure. Thus pharmacokinetic sampling will not be done in this study.

The current proof of concept (POC) Phase 2 trial will evaluate the safety, tolerability, and efficacy of three doses of volixibat (5, 10, and 20 mg) in adult subjects with NASH. Due to the MOA of volixibat, efficacy will be assessed at an interim analysis (IA) by a change of steatosis from baseline to Week 24 compared to placebo (PBO). While the gold standard for quantification of steatosis has historically been an invasive liver biopsy, quantitative magnetic resonance (MR) imaging–based biomarkers for liver fat have evolved rapidly over the last decade, and are increasingly being incorporated into NASH clinical trials. Both MR spectroscopy (S) and MR imaging (I) proton density fat-fraction (PDFF) provide non-invasive means of quantifying intrahepatic lipid content (Reeder et al., 2012). Both techniques have been shown to be accurate, reproducible with a low degree of variability in interpretation, cost-effective and reliable biomarkers of quantitative hepatic fat (Roldan-Valadez et al., 2010; Urdzik et al., 2012; Raptis et al., 2012). Importantly, studies demonstrate a close correlation with steatosis grade histologically (Qayyum et al., 2005; Schwenzer et al., 2009).

In a study of 51 adult subjects with NAFLD, PDFF correlated well with the grade of histologic steatosis, as the mean fat-fraction values of 8.9%, 16.3%, and 25.0% corresponded to histologic steatosis grades 1, 2, and 3, respectively ($P < .0001$) (Permutt et al., 2012). Thus, MRI will be utilized to evaluate the degree of steatosis change from baseline in this study during the IA. The Mozart trial was a randomized, double-blind, PBO-controlled trial of 50 patients with NASH who were randomized to 24 weeks of either ezetimibe or PBO, evaluating the reduction of liver fat by MRI-PDFF as well as by histology. Results revealed that compared to histologic non-responders, histologic responders, defined as a two-point reduction in NAFLD activity score (NAS) without worsening fibrosis, had a statistically significant reduction in net MRI-PDFF of $-4.1\% \pm 4.9$ vs. $+0.6\% \pm 4.1$ ($P <0.036$) with a mean percent change of $-29.3\% \pm 33.0$ vs. $+2.0\% \pm 24.0$ ($P < 0.004$), respectively (Loomba et al., 2015). Thus, in the current trial during the IA, a $\geq 5\%$ steatosis reduction for an active dose compared to PBO will be a clinically meaningful change after 24 weeks of therapy.

Intrahepatic lipid content of less than 1% is considered to be within the normal range (Springer et al., 2015), however, from a study of 2349 people in a general population undergoing MRS, it was concluded that a PDFF value of 5.56% represented the upper limit of the normal range, as determined from the 95th percentile of PDFF in 345 individuals who were not at increased risk for hepatic steatosis (Szczepaniak et al., 2005). Thus, in the current trial, similar to other NASH trials utilizing MR for evaluation, an MRI ≥5% steatosis will be used as an inclusion criterion (Loomba et al., 2015).

1.3 Benefits and Risks

By virtue of volixibat's ability to inhibit ASBT bile acid reabsorption, there is an increase in BA excretion and signaling in the intestine that results in improvements in glucose metabolism and changes in cholesterol and fatty acid synthesis in the liver. Recently, HFD fed mice treated with an ASBTi (SC-435 a surrogate of SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved IR, and decreased NAS (predominantly steatosis). In addition, these HFD-fed mice did not gain weight when treated with SC-435, in spite of consuming increased calories. Finally, the BA pool in these mice changed to predominantly FXR agonist (Rao et al., 2016).

These metabolic actions and preclinical results may prove to be clinically relevant to subjects with NASH.

NASH has recently received considerable attention as awareness of the problem of liver damage and prevalence of the disorder has increased, paralleling the obesity epidemic. Consequences of liver damage are detrimental and can lead to liver failure, hepatocellular carcinoma, and the need for liver transplantation. There is no currently approved medical therapy for NASH. The large unmet medical need and the increased medical resource burden have led to the search for potential therapies to treat NASH.

Nonclinical testing established that the no observed adverse effect level (NOAEL) for volixibat in rats and dogs following 13 weeks of once-daily administration were 1000 and 500 mg/kg/day, respectively. Similarly, testing confirmed that the NOAEL for volixibat in a 6-month study in rats and a 9-month study in dogs were 1000 and 500 mg/kg/day, respectively. In both cases, these were the highest doses tested. Genotoxicity testing has yielded negative findings.

Volixibat is minimally absorbed (Siebers et al., 2017). The pharmacokinetic profiles performed in clinical studies completed to date repeatedly suggest negligible systemic exposure. Furthermore, there has been no observation of clinically relevant changes in fat absorption parameters such as those related to fat-soluble vitamins.

The most frequent TEAEs in the Phase 1 studies were GI and were considered mechanism-based due to elevated BA concentrations in the colon. The percentage of subjects reporting at least 1 TEAE in the GI disorders SOC generally increased with an increasing volixibat dose level (Part 1 Study TDU10632 and Study LUM002-101). Most TEAEs were mild in intensity, and none were assessed as severe.

In the multiple dose studies, the most commonly reported TEAEs in subjects (both healthy and with T2DM) who received volixibat for the longest duration of 28 days in Study LUM002-101 included diarrhea and abdominal pain. The most commonly reported TEAEs in subjects receiving 50 mg volixibat for 14 days (part 3 Study TDR10633) were diarrhea and GI pain.

Overall, there were 2 SAEs (ALT increased and retinal detachment), both of which led to the discontinuation of volixibat. In part 3 of the initial Phase 1 study (TDU10633), 1 subject dosed with 50 mg volixibat for 13 days was withdrawn from the study due to a mild TEAE (which became a SAE due to prolonged hospitalization) of ALT increased that was considered related to volixibat . The subject's ALT level returned to normal after discontinuation of volixibat. A second subject dosed with 10 mg volixibat for 12 days in Study LUM002-101 reported a moderate SAE of ablation of the retina with a bleed in the vitreous body of the right eye that was considered not related to volixibat.

Overall, 3 subjects, all dosed with 5 mg volixibat in Study LUM002-101, discontinued volixibat due to non-serious TEAEs: 1 due to a related TEAE of mild hemorrhagic diarrhea, 1 due to an unrelated TEAE of moderate Epstein-Barr virus infection, and 1 due to mild related TEAEs of diarrhea and anal erosion.

Overall, the observed AEs attributable to volixibat have been self-limited as would be expected given the local MOA of ASBT inhibition in the terminal ileum. Generally, among subjects who experienced GI TEAEs, the events have been mild and diminished over the course of treatment. Please refer to the investigator's brochure for additional information.

Volixibat is a novel drug candidate, demonstrating limited systemic exposure across species with the potential to affect important metabolic pathways associated with NASH. The overall safety, tolerability, and preliminary activity of volixibat in available clinical trials suggest that further investigation is warranted and that there is a positive benefit to risk profile.

Always refer to the latest version of the Volixibat Potassium (SHP626) Investigator's Brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of volixibat.

See Appendix 1 for protocol history, including all amendments.

2 STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Currently there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its MOA and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

2.2 Study Objectives

2.2.1 Primary Objective

To evaluate the effect of volixibat compared to PBO on liver histology

2.2.2 Secondary Objectives

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual NAS components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

2.2.3 Exploratory Objectives

- To explore the effect of volixibat compared to PBO on liver histology (measured by individual SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F))
- To explore the effect of volixibat compared to PBO on anthropometric measures (body weight, body mass index (BMI), waist circumference and waist-hip ratio)
- To explore the effect of volixibat compared to PBO on homeostasis model assessment-IR (HOMA-IR) and HOMA-beta cell function (HOMA-%B) in subjects with T2DM
- To explore the effect of volixibat compared to PBO on BA synthesis (7-alpha-hydroxy-4-cholesten-3-one [C4])
- To explore the effect of volixibat on patient-reported health-related quality of life (HRQoL) and overall health status.

3 STUDY DESIGN

3.1 Study Design and Flow Chart

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio prior to the IA. After randomization of at least 80 subjects

(20/treatment arm), a 24-week IA may eliminate at least one volixibat dose group based on the criteria provided to the Data Monitoring Committee (DMC). Following the IA, enrollment will continue in order to achieve the sample size target of 67 completers in each of the treatment groups that were not dropped at the IA (201 total if 3 arms continue, 134 if 2 arms continue). Accounting for a 20% dropout, 81 subjects will be randomized in each of the treatment groups that continue. Including the projected approximately 49 additional subjects randomized to a dose that is dropped at the IA, 292 subjects (81 for each arm that continues + approximately 49 in the dropped arm) will be randomized if 3 arms (2 volixibat and 1 PBO) continue; 260 subject (81 for each arm that continues + approximately 49 for each of the dropped arms) will be randomized if 2 arms (1 volixibat and 1 PBO) continue. Should the number of subjects randomized prior to the IA to a dose group that is dropped at the IA markedly deviate from the above projection, then the total number of subjects randomized will be adjusted accordingly, not to exceed approximately 324 subjects. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 88 if 1 dose is dropped after the IA and at 78 if 2 doses are dropped after the IA (approximately 30% of the total number of subjects randomized).

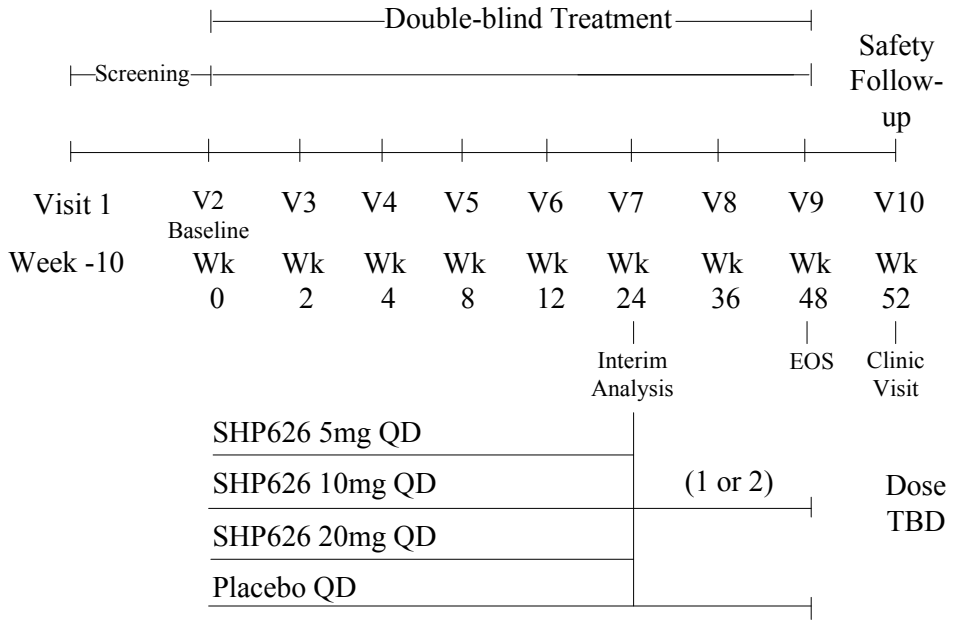
There will be up to 3 study periods (screening, treatment, and follow-up) and an IA. The duration of the Treatment period will be 48 weeks, with an IA at Week 24. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses may be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

The IA will be conducted by an independent DMC after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO.

Once decisions as to dose elimination are made, additional sites may be added. Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining dose groups (noted by "[1 or 2]… Dose TBD" in Figure 1) or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat may be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of investigational product (IP).

The study will be conducted over 3 periods: screening (10 weeks), treatment (48 weeks), and follow-up (4 weeks), with an IA at 24 weeks as outlined in Figure 1.

Figure 1 Study Design Flow Chart



3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 434 days. The last visit is an in-clinic safety follow-up visit. The study will be completed in approximately 4 years.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at approximately 60 to 80 clinical sites in the USA, Canada, and EU.

4 STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and for 30 days following the last dose of the IP as described in Section 4.4.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F0-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met.

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] positive and/or HBVDNA positive; subjects who are hepatitis B core antibody [HBcAb] positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV [defined as HCVAb positive] without a current or prior history of a detectable HCVRNA) may be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the investigational product (IP) (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver biopsy and/or MRI within 6 months of screening, but experienced a weight change of

≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.

5. Contraindications to MRI (eg, claustrophobia, coronary stents, coronary implantable devices, girth, etc.). Stents or other devices may be allowed, at the investigator's discretion, if they do not interfere with the functioning of the MRI machine.
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation (refer to Section 5.2.1, Permitted Treatment).
8. Uncontrolled diabetes defined as HbA1c of ≥9.5% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening, or a sliding scale of insulin is allowed as long as the subject's HbA1c remains < 9.5%. Also refer to Section 5.2.2, Prohibited Treatment.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to liver biopsy, or initiated any time after liver biopsy performed, including the use of total parenteral nutrition (TPN). Also refer to Section 5.2.2, Prohibited Treatment.
11. Serum AST >7 times upper limit of normal (ULN) at screening.
12. Serum ALT >7 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3
15. TB >2 times ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$/L
17. Medical history of impaired hemostasis or use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie 81 mg, aspirin (ASA) or clopidogrel (Plavix) will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.

21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Refer to Section 7.2.3.8, Drug and Alcohol screening. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.
25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior sponsor approval).
31. Subjects who are employees at the unit of the investigational site that is conducting the study.

4.3 Restrictions

Subjects must adhere to the following restrictions for the duration of the study:

- Subjects must remain compliant with inclusion/exclusion criteria.
- Subjects should not become pregnant, father a child, or nurse/breastfeed a baby.
- Subjects should be encouraged to adhere to the same exercise routine and a healthy diet throughout the study.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of child-bearing potential should use an acceptable form of contraception. Females of child-bearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of the IP. If hormonal contraceptives are used they should be administered according to the package insert.

Female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and age ≥51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of child-bearing potential with a negative urine and/or serum β-human chorionic gonadotropin (β-HCG) pregnancy test each study visit. Females of child-bearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of highly-effective contraception.

Acceptable methods of highly-effective contraception (ie, methods that result in a failure rate of <1% per year) are:

- Combined (estrogen and progestogen containing) hormonal contraceptives associated with inhibition of ovulation (oral, intravaginal, transdermal) stabilized for at least 30 days prior to the screening visit (Visit 1) plus a barrier method (eg, condoms or diaphragms with spermicidal gel or foam)
- Progestogen-only hormonal contraception associated with inhibition of ovulation plus a barrier method
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Male sterilization/vasectomized partner
- True abstinence: When this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.)

4.4.2 Male Contraception

Contraception is required for all sexually-active male subjects and their partners. All male subjects (including those who are sterile) agree not to donate sperm, and to use 1 of the following

approved methods of contraception from the baseline visit on Day 0 until 30 days following study discharge:

- Male condom with spermicide
- Sterile sexual partner
- Intrauterine device with spermicide (use by female sexual partner)
- Female condom with spermicide (use by female sexual partner)
- Contraceptive sponge with spermicide (use by female sexual partner)
- Intravaginal system (eg, vaginal ring with spermicide, a diaphragm with spermicide, or a cervical cap with spermicide) (use by female sexual partner)
- Oral, implantable, transdermal, or injectable hormonal contraceptive (use by female sexual partner) plus a barrier method

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from the IP with the CRO Medical Monitor when possible.

If the IP is discontinued, regardless of the reason, the evaluations listed for both Visit 9/end of study (EOS) and Visit 10/Follow-up, are to be performed as completely as possible; however, the EOS liver biopsy will not be required for patients discontinuing prior to Week 44.

Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination, date of stopping the IP, and the total amount of the IP taken must be recorded in the electronic case report form (eCRF) and source documents.

Subjects who discontinue will not be replaced.

4.5.1 Subject Withdrawal Criteria

Medically important events that in the opinion of the investigator, medical monitor or sponsor would compromise the subject's ability to safely continue in the study may result in withdrawal of the subject from the study. Subjects who become pregnant or demonstrate disease progression, defined as the development of signs or symptoms of hepatic decompensation (ie, esophageal variceal hemorrhage, hepatic encephalopathy, ascites, or hepatocellular carcinoma), will be withdrawn from the study and followed as set forth in the protocol.

4.5.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the eCRF. If a subject is withdrawn for more than 1 reason, each reason

should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event (AE)
- Protocol violation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Screen failure
- Noncompliance with study drug
- Physician decision
- Pregnancy
- Progressive disease (refer to Section 4.5.1)
- Study terminated by sponsor
- Other - If "Other" is selected, the investigator must specify the reason on the eCRF

4.5.3 Subjects Lost to Follow-up Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact clinic visit. At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

4.5.4 Safety-related Stopping Rules

Refer to Appendix 4 and Appendix 5 for criteria to assess severity of liver-related AEs and stopping rules for liver-related blood tests, respectively.

An urgent safety review will be conducted within 7 days by the sponsor and in consultation with the DMC if 1 or more of the following criteria are met:

- Death that is considered related to the study drug
- Two SAEs of similar type (defined as same or similar Medical Dictionary for Regulatory Activities (MedDRA) higher level group code), and considered related to the study drug.

The urgent review will be performed by a sponsor safety review group, which will include the study Global Safety Lead (GSL) and the Global Drug Safety (GDS) therapeutic area (TA) Head,

and in consultation with the DMC. The GDS TA Head, not the GSL involved in the study, may be unblinded as part of this urgent safety review, if required. Following the sponsor's review of safety data and consultation with the DMC, one of the following actions will be taken with respect to study status:

- Continue study with protocol unchanged
- Continue study with modifications to the protocol
- Terminate study

Subject safety will be monitored on a continuous basis during this study until the last subject completes his or her last scheduled study visit/assessment.

5 PRIOR AND CONCOMITANT TREATMENT

All non-study treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to signing informed consent at the screening visit (Visit 1) and through the final study contact (including protocol-defined follow-up period) must be recorded on the appropriate eCRF page.

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, and behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate), received within 30 days of the date of first dose of the IP. Prior treatment information must be recorded on the appropriate eCRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of the IP and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate eCRF page.

5.2.1 Permitted Treatment

Medications and supplements including but not limited to vitamin E, betaine, s-adenosyl-l-methionine, ursodeoxycholic acid, milk thistle, gemfibrozil, anti-TNF therapies, probiotics biguanides (metformin), thiazolidinediones (TZDs) and GLP-1 RAs that have been used to treat NAFLD/NASH are allowed during the course of the study if the subject has been on a stable dosing regimen (ie, same dose and frequency in the previous 6 months prior to the qualifying liver biopsy and not initiated after qualifying liver biopsy) and will continue this dosing regimen throughout study participation. Use of antiplatelet medications is also allowed. The investigator must contact the CRO medical monitor to discuss any changes to concomitant medications that may impact the study.

5.2.2 Prohibited Treatment

The following list of prohibited drugs cannot have been taken for more than 2 weeks within the 6 months prior to randomization and are excluded while on study.

- Systemic Glucocorticoids
- Tamoxifen
- Amiodarone
- Methotrexate
- Alcohol (see Section 4.2)
- Griseofulvin
- Total parenteral nutrition
- Obeticholic acid
- Valproate
- Nucleoside Analogues (except acyclovir)
- Tetracycline (high dose; >1g)
- Estrogens at doses greater than 2 mg QD used for hormone replacement
- Anabolic steroids
- Bile acid sequestrants such as cholestyramine or colestipol
- Any other known hepatotoxins including over-the counter therapies and herbal therapies such as germander, chaparral and ma-huang.

This is not a comprehensive list. Treatments not listed above are generally considered allowable, unless considered a potential hepatotoxin. Antidiarrheals will be allowed at the discretion of the investigator, with the exception of BA sequestrants.

6 INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is volixibat potassium (volixibat), which will be provided in 5, 10 and 20 mg capsule form. Additional information is provided in the current Volixibat Potassium (SHP626) Investigator's Brochure.

The reference/comparator product is an identical PBO which will be provided in capsule form.

6.1.1 Blinding the Treatment Assignment

The IP will be supplied as double-blind blister packs. The actual double-blind treatment given to individual subjects is determined by a randomization schedule which will be automatically assigned by the interactive response technology (IRT). Placebo capsules, which exactly match the IP, will be used in the blister packs to provide the same number and size capsules for each of the doses within the treatment groups.

6.2 Administration of Investigational Product(s)

All IP and supplies will be provided by Shire or its designee**.** At each visit, subjects will be supplied with enough IP to last until the subsequent visit. Lost or damaged IP will be replaced as needed. Volixibat will be supplied to the clinical research center (CRC) as powder in capsule. Volixibat will be supplied in identical capsules in strengths of 5, 10, and 20 mg (with matched PBO).

6.2.1 Interactive Response Technology for Investigational Product Management

IRT will be used for the following investigational tasks:

- Randomization
- Supply management
- Inventory management and supply ordering
- Expiration tracking
- Returns
- Emergency unblinding

6.2.2 Allocation of Subjects to Treatment

This is a double-blind, PBO-controlled study. The actual treatment given to individual subjects is determined by a randomization schedule.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation.

The randomization number represents a unique number corresponding to the IP allocated to the subject, once eligibility has been determined.

Individual subject treatment is automatically assigned by the IRT.

Once a randomization number/unique identifier has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. If a randomization number/unique identifier is allocated incorrectly, the clinical research associate (CRA)/study monitor must be notified as soon as the error is discovered.

IP packaging identification numbers, separate from randomization numbers/unique identifiers, may also be assigned to subjects for specific treatment assignment as dictated by the study. In these cases, the same IP packing identification number may not be assigned to more than 1 subject.

Subjects will be equally allocated to all volixibat doses and PBO if their randomization precedes the 24 week IA. If a subject's randomization occurs after the 24 week IA, subjects will be equally allocated to all remaining volixibat doses (determined based on the results of the IA) and PBO. The randomization will be stratified by baseline T2DM and NAS ≥6 or NAS = {4,5}. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 88 if 1 dose is dropped after the IA and at 78 if 2 doses are dropped (approximately 30% of the total number of subjects randomized).

6.2.3 Dosing

All doses of volixibat or matching PBO will be administered orally as a capsule in a double-blinded fashion. The first dose of IP for each subject will be administered in the clinic. The dose will be administered with 240 mL of water and should be given 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat. The subject should make all attempts to consistently take the IP around the same time each day. If a dose is missed at the normally scheduled time, the subject can make up the dose that day as long as no more than 1 dose is taken in a 10-hour period.

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be broken during the study except in emergency situations where the identification of the IP is required for further treatment of the subject. The investigator should contact the CRO medical monitor and the Shire study physician at the same time and as soon as possible after the investigator has been unblinded.

In the event that the treatment assignment is broken, the date and the signature of the person who broke the code are to be recorded in the source documents and the IRT, and the reason for breaking the code will be recorded in the source documents and the clinical database. Upon breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code-breaks that occur must be reported to the CRO medical monitor. Code-break information is held by the pharmacist/designated person at the site and by the CRO medical monitor for the study or designee.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled with a minimum of the protocol number, medication identification number, dosage form (including product name and quantity in pack), directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements 'For clinical trial use only', and/or 'CAUTION: New Drug - Limited by Federal (or USA) Law to Investigational Use', 'Keep out of reach of children', and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Additional labels may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The sponsor or designee will provide the IP for this study. The IP is packaged in the following labeled containers:

- Volixibat 5 mg capsules
- Volixibat 10 mg capsules
- Volixibat 20 mg capsules
- Volixibat PBO capsules

6.3.3 Storage

The investigator has overall responsibility for ensuring that the IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented.

IPs are distributed by the pharmacy or designated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP labels as they are distributed.

All IP must be stored at the clinic at 20 - 25^{o}C (68 - 77^{o}F); excursions are allowed between 15 - 30^{o}C (59 - 86^{o}F).

IPs must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the IP is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion below 15^{o}C (59^{o}F) or above 30^{o}C (86^{o}F); these excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the product be

dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room or a change in storage location.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing the IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed IP will be documented on the eCRFs and/or other IP record. The investigator is responsible to ensure the retrieval of all study supplies from subjects.

No IP stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records. The site must ensure that the accountability and destruction records are complete, accurate, and ready for verification at each monitoring visit.

The process for return and destruction of IP must be determined and documented during the study start-up phase.

With the written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject returned IP, and empty/used IP packaging may be destroyed at the site or a local facility on an ongoing basis throughout the study and at the end of the study following verification of accountability by the CRA/study monitor. In this case, destruction records identifying what was destroyed, when, how, and by whom, must be obtained with copies provided to the sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Alternatively, in the absence of written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject-returned IP, and empty/used IP packaging may be required to be sent to a nominated contractor on behalf of the sponsor for IP destruction on an ongoing basis throughout the study and at the end of the study. IP being returned to the sponsor's designated contractors also must be counted and verified by clinical site personnel and the CRA/study monitor. For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, IRT) do not require a shipment form. Returned IP must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any IP prior to shipment. Shipment of all returned IP must comply with local, state, and national laws.

6.5 Subject Compliance

Subjects must be instructed to bring their unused IP and empty/used IP packaging to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, blister pack) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details on the drug accountability form.

7 STUDY PROCEDURES

7.1 Study Schedule

This study will consist of a screening period of up to 70 days (the screening period may be minimally extended under special circumstances only with the explicit approval of the Shire study physician, a 48-week Treatment period, and a Follow-up visit 4 weeks after treatment ends. A detailed display of all study procedures is provided in Table 1.

Patients will be assessed according to the following schedule:

- Screening – Visit 1 (Weeks -10 to 0 [Day -70 to Day -1])
- Baseline – Visit 2 (Week 0 [Day 0])
- Treatment and Assessments – Visits 3 through 9 (Week 2 through Week 48)
- Follow-up – Visit 10 (Week 52, 4 weeks after completion of dosing)

7.1.1 Screening (Visit 1)

Screening procedures must be completed within 70 days prior to randomization for the first dose of the IP. The screening period may be minimally extended under special circumstances only with the explicit approval of the Shire study physician. At the screening visit, considered Visit 1 (Week -10 to 0, Day -70 to -1), all screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from the subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

The following screening procedures should be assessed at the beginning of the visit window, preferably between Day -70 and Day -49: inclusion/exclusion criteria, collection of subject information (demographics, medical and medication history AEs, and concomitant medications), a physical examination including vital signs, height, weight, and waist/hip measurements; collection of blood and urine samples for screening and safety assessments, performance of an electrocardiogram (ECG), and scheduling of MRI and liver biopsy. MRI and liver biopsy (if one has not been completed in the previous 6 months) should be performed with sufficient time to ensure results are received prior to the baseline visit.

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered the IP. A subject who had been designated a screen failure may be re-screened up to one time. However, re-screened subjects must begin the screening procedure again, must be re-consented, and will be assigned a new subject number. Additionally, a subject's abnormal screening lab results may be repeated once for confirmation before designating a subject as a screen failure.

An investigative site may provide additional liver biopsy tissue to the central reader to be evaluated when the following criteria are met: 1.) the subject qualifies for the study on all inclusion/exclusion parameters except for liver histology, and 2.) the investigator's clinical assessment of the subject's local liver histology differs from that of the centrally read results, and 3.) the request to provide more tissue to the central reader for a second read must be requested before the patient is declared a screen failure. Any additional tissue samples provided must be read by the central reader and the central reader determines the final histological assessment.

7.1.2 Baseline (Visit 2)

Following the screening visit, subjects will return to the clinic within 70 days for Visit 2, (considered Week 0, Day 0), for baseline assessments including review of the inclusion/exclusion criteria, adverse events, and concomitant medications; physical examination with vital signs, weight, and waist/hip measurements; ECG; collection of blood and urine samples; stool assessment; and completion of the patient-reported outcome (PRO) EuroQol-5 Dimension-5 Level Questionnaire (EQ-5D-5L) survey. After completion of these assessments, subjects are randomized using Interactive Response Technology (IRT) to 1 of 4 treatment arms receiving volixibat 5 mg, 10 mg, or 20 mg, or PBO before the IA and then to 1 of the remaining arms after the IA. The initial supply of study drug is dispensed to ensure adequate daily dosing until the next scheduled study visit.

7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)

During the 48 weeks of double-blind treatment, 7 clinic visits are scheduled to occur as follows:

- Visit 3 - Week 2, Day 14 (+/- 3 days)
- Visit 4 - Week 4, Day 28 (+/- 3 days)
- Visit 5 - Week 8, Day 56 (+/- 5 days)
- Visit 6 - Week 12, Day 84 (+/- 5 days)
- Visit 7 - Week 24, Day 168 (+/- 7 days) – Interim Analysis
- Visit 8 - Week 36, Day 252 (+/- 7 days)
- Visit 9 - Week 48, Day 336 (+/- 7 days) – End of Study

Subjects are reminded not to eat and not to take their study drug on the day of scheduled study visits prior to completion of assessments. They should bring their study drug with them to the visit. IP should be taken 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat.

The Schedule of Assessments provided in Table 1 details the procedures to be completed at each visit. All Treatment visits (3 through 9) will include weight and waist/hip measurements, assessment of vital signs, and blood sampling for completion of biochemistry, hematology, serum glucose, lipid panel, HbA1c, and serum liver-related blood tests. At all visits, adverse events, and concomitant medications will be collected for all subjects, and female subjects of childbearing potential will have a urine pregnancy test. Subjects will return containers of unused study drug for assessment of accountability and compliance which will be documented in the IRT. New supplies of study drug will be dispensed at Visits 3 through 8.

Additional assessments will also occur less frequently during the Treatment Period for vitamin A, vitamin D, vitamin E, vitamin K via PT/INR, insulin, thyroid testing, urinalysis, ECG, physical examinations, stool assessment, and completion of the EQ-5D-5L.

An MRI will be repeated at Visit 7 for the 24-week IA and at Visit 9 (Week 48) for all subjects. A final liver biopsy will be performed at Visit 9 (Week 48) for all subjects unless a subject discontinues prior to Week 44, in which case the EOS liver biopsy is not required.

Sites that have supplied local slides for central reading for subject eligibility at Screening must also provide local liver histology slides for the subject to be centrally read for the Week 48 EOS liver biopsy. The central reader will provide the final liver histology assessment for Week 48.

7.1.4 Follow-up (Visit 10)

The follow-up period for this protocol is 4 weeks after the last dose of study drug with a final Follow-up visit scheduled for Week 52. Procedures to be completed at this final visit include physical examination, weight, waist/hip measurements, vital signs, samples for biochemistry and hematology, serum glucose, lipid panel, HbA1c, serum liver-related blood tests, insulin, thyroid

testing, stool assessment, and urine pregnancy test (for women of childbearing potential). Adverse events and concomitant medications will be recorded. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.5 Additional Care of Subjects after the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

All assessments and procedures are to be performed by the Investigator or a qualified designee who has been trained in the protocol. Assessments are to be performed according to the schedules shown in Table 1. If a subject terminates the study early, the CRC will make reasonable effort to perform the EOS and safety follow-up assessments and procedures for the subject's safety and well-being.

7.2.1 Demographic and Other Baseline Characteristics

Demographic details will be obtained at screening and recorded on the eCRF. Data collected will include age, gender, ethnicity, height, and weight.

7.2.2 Efficacy

7.2.2.1 Liver Biopsy

Liver biopsies will provide histologic data for confirmation of the diagnosis of NASH, assessment and grading of NASH activity, and scoring of steatosis, lobular inflammation, ballooning, as well as fibrosis and additional features (see Appendix 2 and Laboratory Manual for additional information).

7.2.2.2 MRI

The presence of ≥5% steatosis on screening MRI from a centrally read radiologist will be performed either during the screening period or within 6 months prior to the first visit. Steatosis is assessed by MRI hepatic fat fraction. The Week 24 MRI will be conducted for subjects in the IA set only.

7.2.3 Safety

7.2.3.1 Medical History and Medications

Medical history including important medical events and concomitant medication and illnesses will be obtained at screening and will be recorded on the eCRF. Any existing medical condition present prior to the time of randomization should be reported as medical history

7.2.3.2 Physical Examination

A complete physical examination will be performed with a thorough review of body systems at screening, baseline prior to randomization, and at study visits specified in Table 1. Physical

examinations will include a review of the subject's general appearance, as well as evaluation of the body systems including:

- Eyes, ears, nose, throat
- Lymph nodes
- Cardiovascular
- Skin
- Abdomen
- Neurological
- Spine and extremities

Abnormalities identified at the screening visit (Visit 1) will be documented in the subject's source documents and on the medical history eCRF. Changes after the screening visit (Visit 1) will be captured as AEs on the AE eCRF page, if deemed clinically significant by the investigator.

Height will be measured at the screening visit only while weight and waist and hip circumference will be recorded at all study visits. BMI will be calculated programmatically.

7.2.3.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.3.4 Vital Signs

Vital signs include oral, temporal, or tympanic temperature, sitting blood pressure, pulse, and respiratory rate. Blood pressure should be determined by cuff (using the same method, the same arm, and in the same position throughout the study). Any deviations from baseline (Visit 2) vital signs which are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

7.2.3.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the central laboratory's normal procedures. Reference ranges supplied by the central laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Clinically significant findings should be evaluated for recording as adverse events on the eCRF. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical

condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Blood urea nitrogen (BUN)
- C4
- Calcium (Ca)
- Bicarbonate (CO2)
- Chloride (Cl)
- Creatinine
- Creatine kinase
- Gamma glutamyl transferase (GGT)
- Glucose
- Lactate dehydrogenase (LDH)
- Magnesium (Mg)
- Phosphate (P)
- Potassium (K)
- Sodium (Na)
- Total and direct bilirubin
- Total cholesterol
- Protein
- Triiodothyronine (T3)
- Thyroid-stimulating hormone (TSH)
- Triglycerides
- Uric acid

Hematology

- Hemoglobin
- Hematocrit
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Platelets
- Red blood cell (RBC)
- White blood cell (WBC) count with differential
- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Urinalysis

- Appearance (clarity and color)
- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopic examination of sediment
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen
- Oxalate

7.2.3.6 Additional Laboratory Assessments

Laboratory samples will be collected and assessed for:

- HIV testing and assessment of Hepatitis B/C including HBcAb, HBsAg, HBVDNA, and HCVAb (if HCVAb is positive an HCVRNA will be performed) will occur at the screening visit only
- Lipid panel including fasting total cholesterol, HDL-C, LDL-C, and triglycerides at each scheduled study visit (Visits 1 through 10)
- HbA1c will be tested at every scheduled visit (Visits 1 through 10)
- Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points at Visits 4, 7, and 9
- Insulin will be tested at screening, baseline, at Visit 4 and Visits 6 through 10
- HOMA-IR and HOMA-%B: will be calculated programmatically from serum glucose tested at screening, baseline, Visits 7, 9, and 10
- Vitamin A, Vitamin D, Vitamin E, and Vitamin K (via PT/INR) will be tested at baseline, and Visits 4, 7, and 9.
- Thyroid testing including TSH and T3 will be tested at screening, baseline, and Visits 4, 6, 7, 9, and 10
- C4 samples will be collected at baseline and Visits 6, 7, 9, and 10

7.2.3.7 Pregnancy Test

A urine pregnancy test is performed on all females of child-bearing potential at the screening visit (Visit 1), baseline visit (Visit 2), at each Treatment visit (Visits 3 through 9) and at the Final visit (Visit 10), or if pregnancy is suspected, or on withdrawal of the subject from the study. A positive urine pregnancy test must be followed with a serum pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Section 8.1.7.

7.2.3.8 Drug and Alcohol Testing

A urine screen for drugs of abuse and blood test for alcohol will be performed at screening and baseline as described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the eCRF database.

Any positive result for drugs of abuse at the screening or baseline visits may exclude the subject from further participation in the study. A positive test for drugs of abuse or alcohol done at the investigator's discretion discovered at any time during the study will be grounds for study discontinuation.

7.2.3.9 Electrocardiogram

An ECG (12-lead) will be performed at the times specified in Table 1 in accordance with the clinical site's standard practice(s) and equipment supplied by the CRC. Recordings of ECGs will be read locally at the clinical site by the investigator or designee. The ECG will include assessments of heart rate and PR, RR, QRS, and QT intervals. Identification of any clinically significant findings and/or abnormalities will be recorded on the eCRF.

7.2.3.10 Health-related Quality of Life Assessments

The EuroQol (EuroQol. 2016. Available at: http://www.euroqol.org. [Accessed 17 February 2016]) EQ-5D-5L ("EQ-5D") is a widely used standardized questionnaire that assesses generic HRQoL and is also recommended for health-economic evaluations. The EQ-5D includes two components: a descriptive profile and a visual analogue scale (VAS). The descriptive profile includes five dimensions (ie, pain/discomfort, mobility, usual activities, self-care and anxiety/depression), each with five levels (ie, no problems, slight problems, moderate problems, severe problems, extreme problems). An EQ-5D index can also be derived from the data which summarizes health status using a single value (ie, health-state utility). The psychometric properties of the EQ-5D-5L have been established and well documented (see Appendix 3).

7.2.3.11 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 146 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 146 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8 ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not the IP is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE eCRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of the IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of the IP, but the dyspepsia becomes severe and more frequent after first dose of the IP has been administered, a new AE of severe dyspepsia (with the appropriate date of onset) is recorded on the appropriate eCRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Appendix 4 provides criteria to assess the severity of liver-related adverse events.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to the IP for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the IP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the eCRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown.

8.1.4 Symptoms of the Disease Under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation of the disease and are part of the efficacy data to be collected in the study; however, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pre-treatment value observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.6 Criteria for Discontinuation of Treatment

Appendix 5 provides guidelines for discontinuation of treatment based on elevated ALT, AST, TB, and associated signs and symptoms.

8.1.7 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.4.

Any report of pregnancy for any female study participant (or the female partner of a male participant) must be reported within 24 hours to the Shire Global Drug Safety (formerly Pharmacovigilance and Risk Management) Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO medical monitor and the Shire study physician using the details specified in the Emergency Contact Information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days postpartum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event

and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-HCG test or ultrasound result will determine the pregnancy onset date.

8.1.8 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of the IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society
- Misuse – Intentional use of the IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol)
- Overdose – Intentional or unintentional intake of a dose of an IP exceeding a pre-specified total daily dose of the product.
- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the Volixibat Potassium (SHP626) Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Drug Safety (formerly Pharmacovigilance and Risk Management) Department and the Shire study physician within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.8) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Drug Safety Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the Shire study physician using the details specified in the Emergency Contact Information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence (whether considered to be related to the IP or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may

require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. 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.

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.4, and must be reported to the Shire Global Drug Safety Department and the Shire study physician within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Drug Safety Department within 24 hours of the first awareness of the event.

8.2.4 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.5 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received the IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

8.2.6 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The CRO is responsible for notifying the relevant regulatory authorities as appropriate: USA central IRBs/EU central ECs of related, unexpected SAEs.

In addition the CRO is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP626-201 program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9 DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the eCRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Data that may potentially unblind the treatment assignment (eg, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513).

9.5 Planned Interim Analysis and Data Monitoring Committee

9.5.1 Planned Interim Analysis

The IA will be conducted after at least 80 subjects have received 24 weeks of treatment. To protect study integrity, the IA will be performed by an independent statistician and statistical reporting group as well as a gastroenterologist and hepatologist who are not involved in the clinical trial conduct and are not responsible for the final analysis of clinical trial data. The IA will be performed to evaluate the safety, tolerability, and efficacy of 3 doses of volixibat each compared to PBO and may be used to drop 1 or 2 doses of volixibat, or to terminate the trial.

The criteria for deciding whether or not to continue randomizing to a particular volixibat dose following the Week 24 IA will be specified in the DMC charter. In general, randomization to any volixibat dose determined to be unsafe, intolerable or ineffective/futile at the IA will be discontinued. If all of the volixibat doses are determined to be intolerable or ineffective/futile the clinical trial will be terminated. If none of the volixibat doses are determined to be unsafe, intolerable or ineffective/futile then randomization to the volixibat dose that is the least promising or least helpful in characterizing the dose-response relationship (as specified in the DMC charter) may be discontinued. Tolerability will be assessed by comparing the number of discontinuations due to any one TEAE (presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain) between each volixibat dose group and PBO group. Efficacy will be assessed based on a reduction of steatosis (assessed by MRI-PDFF) and ALT by comparing the change from baseline in MRI-PDFF and percent change from baseline in ALT at Week 24 between each volixibat dose group and PBO group.

Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. That is, a subject receiving a dose to which randomization is stopped after the IA will continue to receive that same dose throughout the course of the study, and complete all required assessments, unless a dose is determined to be unsafe, in which case the affected subjects would be discontinued from the study and followed as per Section 4.5. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining volixibat dose groups or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or two doses of volixibat may be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of IP.

9.5.2 Data Monitoring Committee

An independent DMC will be established to assess safety, tolerability, and efficacy during the study, as well as to ensure the validity and scientific merit of the trial. The DMC will monitor ongoing data generated by the study at regular intervals for the duration of the study. Their role is to protect the interests of the subjects in the study and of those still to be entered, by review of

accumulating data generated in the study. If a safety concern is identified (eg, if either of the criteria are met in Section 4.5.4, Safety-related Stopping Rules), the DMC may recommend an action to the sponsor on further study conduct, including stopping the study at any time.

In addition, the DMC will review the results of the Week 24 IA and make recommendations concerning study discontinuation due to intolerability or futility, or the continued randomization of 1 or more volixibat dose groups and PBO.

The roles, responsibilities, and rules governing operation of the DMC will be discussed in full in a DMC charter. The DMC charter will define the primary responsibilities of the DMC; guide its activities, its relationship with other study components, its membership, and the purpose and timings of its meetings. It will provide the procedures for ensuring confidentiality, formal communication, and outline of the content of reports that will be provided by the DMC.

Appropriate summary statistics and data listings will be provided to the DMC by an independent statistician supported by an independent statistical reporting group not otherwise assigned to the study.

The recommendations made by the DMC to alter the conduct of the study or to stop the study will be forwarded to Shire for final decision. The implementation of any DMC recommendation is solely the responsibility of the sponsor. Shire will forward such decisions to regulatory authorities, as appropriate.

9.6 Sample Size Calculation and Power Considerations

These are the primary hypotheses that are being tested in this study:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.

- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

Response rates of 21% (PBO) and 45% (active) were reported in the FLINT trial (Neuschwander-Tetri et al., 2015) using the same primary end-point of reduction of NAS by at least two points (without worsening of fibrosis). Based on those response rates, 67 subjects per group completing the trial are needed for 80% power with a 10% type I error. Holm multiplicity adjustment was included in the sample size estimate.

Approximately 899 subjects will be screened to randomize 292 subjects to achieve 201 completers in the three arms (2 volixibat and 1 PBO) that continue after the IA (one dose is dropped at the IA); approximately 807 subjects will be screened to randomize 260 subjects to achieve 134 completers in the two arms (1 volixibat and 1 PBO) that continue after the IA (two

doses are dropped at the IA). After randomization of at least 80 subjects (20/treatment arm), a 24-week IA may eliminate at least one active dose group based on the criteria provided to the DMC. The sample size target for this study is 67 completers in each of the treatment groups that were not dropped at the IA (201 total if 3 arms continue, 134 if 2 arms continue). Accounting for a 20% dropout, 81 subjects will be randomized in each of the treatment groups that continue. Including the projected approximately 49 additional subjects randomized to a dose that is dropped at the IA, 292 subjects (81 for each arm that continues + 49 in the dropped arm) will be randomized if 3 arms (2 volixibat and 1 PBO) continue; 260 subject (81 for each arm that continues + 49 for each of the dropped arms) will be randomized if 2 arms (1 volixibat and 1 PBO) continue. Should the number of subjects randomized prior to the IA to a dose group that is dropped at the IA markedly deviate from the above projection, then the total number of subjects randomized will be adjusted accordingly, not to exceed approximately 324 subjects.

9.7 Study Population

The Screened Set will consist of all subjects who have signed an informed consent.

The Randomized Set will consist of all subjects in the Screened Set who have been randomized into the study.

The Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an AE or reporting the absence of AEs).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

The Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the data cut time of the IA.

9.8 Efficacy Analyses

All efficacy analyses will be based on the FAS and all statistical tests will be 2-sided hypothesis tests performed at the 10% level of significance on those doses continued after the IA, accounting for the number of doses dropped at the IA. Also, all confidence intervals will be 2-sided confidence intervals, unless otherwise stated.

9.8.1 Primary Efficacy Endpoint

The primary endpoint is the binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points, without worsening of fibrosis, from baseline NAS.

The FAS will be used to assess the primary efficacy endpoint. The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS

will be analyzed. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of T2DM at baseline and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Subjects with missing data for the primary efficacy endpoint at the Week 48 visit will be considered as non-responders. Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level.

Analyses which explore the impact of the missing data on the primary efficacy endpoint will be conducted. These analyses may compare imputations of the missing values which favor the PBO and/or imputations which favor the active. In addition, analyses utilizing existing HFF and/or ALT data may be performed. Further, if the pattern of missing values does not appear uniformly distributed among the treatment arms, imputation method(s) based on informative missingness may also be performed. Analyses investigating the impact of missing data will be detailed in the Statistical Analysis Plan.

9.8.2 Secondary Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the secondary efficacy endpoints. Binary secondary efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous secondary efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
- Change from baseline to Week 48 on hepatic steatosis as measured by MRI-PDFF.
- Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH CRN)
- Resolution of NASH (defined as total absence of ballooning [score = 0], absent or mild inflammation [score 0-1], steatosis can be present [score 0-3]) without worsening of fibrosis as assessed by liver histology at Week 48.
- Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
    - alanine aminotransferase (ALT)
    - aspartate aminotransferase (AST)
    - alkaline phosphatase (ALP)
    - gamma glutamyl transferase (GGT)
    - total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
    - fasting serum glucose levels

  - insulin levels
  - HbA1c
- Change from baseline to Week 48 on serum lipids measured by:
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

9.8.3 Exploratory Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the exploratory efficacy endpoints. Binary exploratory efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous exploratory efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F)
- Change from baseline to Week 48 on anthropomorphic measures:
  - body weight
  - BMI
  - waist circumference
  - waist-hip ratio
- Change from baseline to Week 48 in subjects with T2DM on homeostasis measured by:
  - Homeostasis model assessment -IR (HOMA-IR)
  - HOMA-beta cell function (HOMA-%B)

9.9 Safety Analyses

The Safety Analysis Set will be used to assess the safety endpoints.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. Treatment-emergent AEs are defined as AEs that start or worsen on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be calculated by SOC, by preferred term, and by treatment group. Treatment-emergent AEs will be further summarized by severity and relationship to the IP. Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

9.10 Other Analyses

9.10.1 Health-related Quality of Life Analyses

- Change from baseline to Week 48 on the EQ-5D index (Appendix 3). The EQ-5D index will be derived using published UK-based preference weights. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.
- Change from baseline to Week 48 on the EQ-5D VAS score. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.
- Change from baseline to Week 48 in the proportion of subjects reporting having problems (none, slight, moderate, severe, extreme) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire. Proportions and 95% confidence intervals will also be generated for the baseline and Week 48 values as well as for change from baseline.

9.10.2 Stool Assessment

Stool hardness and number of evacuations will be assessed throughout the study. Stool hardness will be assessed for the softest evacuation within 24 hours of each clinic visit using the Bristol Stool Chart, a medical aid designed to classify the form of human feces into 7 categories where Type 1 is the hardest and Type 7 is the softest. Appendix 6 provides a sample of the Bristol Stool Chart. Number of evacuations within the past 24 hours prior to the clinic visit will be recorded at specified times as per Table 1. Descriptive statistics by treatment group will be presented for each time point at which the variable is measured.

10 SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements. Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the Declaration of Helsinki.

The name and address of each third party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all

applicable industry regulations, ICH GCP Guideline E6 (1996) and E6 R2 (2017), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of the IP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

10.1.3 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

10.1.5 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.do

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996) and E6 R2 (2017), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all the IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for

multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

Study eCRFs are supplied by the sponsor and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. Study eCRFs must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

All eCRF data must be endorsed by the investigator.

The CRA/study monitor will verify the contents of the eCRF against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, subject diary cards, original clinical laboratory reports, and histology and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, images, etc.). Non-study site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other USA national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in the IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB/EC and which received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor, prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an EC opinion can be the sponsor, the investigator, or for multicenter studies the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the sponsor or its designee has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor, the investigator or for multicenter studies the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All USA-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the sponsor or its designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market volixibat; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results / Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee, which is done for large, multicenter Phase 2-4 and certain other studies as determined by Shire. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 60 days prior to submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

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12 APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	24 February 2016	Global
Amendment 1	8 April 2016	Global
Amendment 2	19 July 2016	Global
Amendment 3	22 March 2017	Global
Amendment 4	25 August 2017	Global

APPENDIX 2 FATTY LIVER DISEASE HISTOLOGY SCORING

Diagnosis: NASH, Suspicious/Borderline NASH (type a or b), NAFLD, Not NAFLD

NAS SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Lobular Inflammation	0	no foci
		1	< 2 foci per 200x field
		2	2-4 foci per 200x field
		3	> 4 foci per 200 x field
	Ballooning	0	None
		1	Rare or diagnostically borderline
		2	Many or Prominent ballooned hepatocytes

SAF SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Ballooning	0	normal hepatocytes with cuboidal shape and pink cytoplasm
		1	clusters of hepatocytes with rounded shape and pale cytoplasm usually reticulated
		2	grade 1 plus enlarged hepatocytes, 2 x bigger than normal cells.
	Lobular Inflammation	0	none
		1	< 2 foci per 200x field
		2	>2 foci per 200x field

Steatosis 1,2,3 + Ballooning 1,2 + Lobular Inflammation 1,2 = NASH
Steatosis 1,2,3 +Ballooning 0 + Lobular Inflammation 0,1,2 = NAFLD
Steatosis 1,2,3 + Ballooning 1,2 + Lobular 0 = NAFLD
Steatosis 0 = no NAFLD

FIBROSIS SCORE	None	0
	Mild zone 3 perisinusoidal (requires trichrome)	1a
	Moderate Zone 3 perisinusoidal (visible on H&E)	1b
	Portal/periportal only	1c
	Portal, periportal and perisinusoidal	2
	Bridging	3
	Cirrhosis	4

References: Kleiner et al., 2005; Bedossa, 2012

ADDITIONAL SCORING FEATURES

Expanded Balloon Score

0: None

1: Few Non classic

2: Few Classic

3. Many Classic

4: Severe Classic

Portal Inflammation

0: None

1: Minimal

2: Mild

3: More than Mild

Megamitochondria

0: None

1: Present

Acidophil Bodies

0: None or rare

1: Present

Fibrosis location if ≥ stage 2

Portal Predominant

Central Predominant

No predominance

Steatosis Zone

Zone 1 predominant

Zone 3 predominant

Azonal

Panacinar

Glycogenosis

None

Focal

Diffuse

APPENDIX 3 EQ-5D-5L HEALTH QUESTIONNAIRE UK SAMPLE ONLY, NOT FOR OFFICIAL USE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

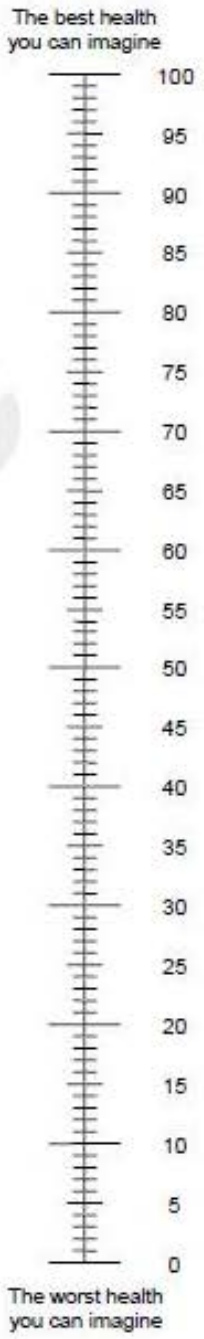
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 4 CRITERIA TO ASSESS SEVERITY OF LIVER-RELATED ADVERSE EVENTS

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
ALT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
AST	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Alkaline Phosphatase	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
GGT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Bilirubin	Normal	>1.0-1.5	>1.5-2.5	>2.5-5	>5

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
Note: Values expressed as multiples of the upper limit of the normal range (ULN).

APPENDIX 5 STOPPING RULES FOR LIVER-RELATED BLOOD TESTS

Treatment-Emergent ALT	Treatment-Emergent TBL	Symptoms	Action
Normal baseline[a]: ALT >5 × ULN Elevated baseline: ALT >3 × baseline or >300 U/L (whichever occurs first)	Normal For patients with Gilbert's syndrome: No change in baseline TBL	None	Repeat ALT, AST, ALP, TBL, in 2–5 days Follow-up for symptoms.
Normal baseline: ALT >8 × ULN Elevated baseline: ALT >8 × baseline or >500 U/L (whichever occurs first)	Normal Patients with Gilbert's syndrome: No change in baseline TBL	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >5 × ULN Elevated baseline: ALT >3 × baseline or >300 U/L (whichever occurs first)	TBL >2 × ULN For patients with Gilbert's syndrome: Doubling of direct bilirubin	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >5 x ULN Elevated baseline: ALT >3 x baseline or >300 U/L (whichever occurs first)	Normal or elevated	i.e. Severe fatigue, nausea, vomiting, right upper quadrant pain	Interrupt study drug. Initiate close monitoring and workup for completing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.

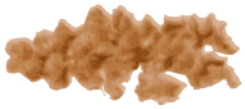
ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; TBL=total bilirubin; ULN=upper limit of normal.

[a]Baseline is Visit 2

Source: Chalasani and Regev, 2016.

APPENDIX 6 BRISTOL STOOL CHART

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

PROTOCOL: SHP626-201

TITLE:	A Phase 2 Double-blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)
DRUG:	Volixibat potassium (SHP626)
IND:	123,847
EUDRACT NO.:	2016-000203-82
SPONSOR:	Shire Human Genetic Therapies, Inc. 300 Shire Way Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Philip Newsome MD Director of Centre for Liver Research University of Birmingham Edgbaston, Birmingham B15 2TT UK
NATIONAL/ COORDINATING INVESTIGATOR/UK	Guruprasad P Aithal MBBS, MD, FRCP, PhD Head of the Division, Nottingham Digestive Diseases Centre Director, NIHR Nottingham Digestive Diseases Biomedical Research Unit Nottingham University Hospitals and University of Nottingham Queen's Medical Centre Nottingham NG7 2UH
PROTOCOL HISTORY:	Amendment 3: 22 March 2017 Amendment 2: 19 July 2016 Amendment 1: 8 April 2016 Original Protocol: 24 February 2016

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature:	**Date:** 24 Mar 2017
Melissa Palmer, MD, FAASLD Global Clinical Development Lead (GCDL) - Hepatology	

Investigator's Acknowledgement

I have read this protocol for Shire Study SHP626-201.

Title: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS VERSION

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **3**	**Amendment Date** **22 March 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Study Synopsis	Extended planned study period from July 2019 to July 2020.	• To accommodate a longer enrollment period.
Study Synopsis	Revised 1) Eligibility criteria; 2) sample size description; and 3) definition of secondary endpoint "Resolution of NASH"	• To improve clarity • To align with changes in the study protocol body.
Table 1 Schedule of Assessments, Section 7.2.3.4 Vital Signs	Revised footnote "g" and text to include temporal temperature measurement.	• To allow for collection of temperature temporally.
Table 1 Schedule of Assessments	Revised footnote "h" to describe the process for providing additional liver biopsy tissue to the central reader.	• To allow additional liver tissue to be sent to the central reader.
Table 1 Schedule of Assessments	Revised footnote "k" to clarify that subjects are not required to have Vitamin A, D, and E testing at the screening visit.	• To eliminate additional blood tests.
Table 1 Schedule of Assessments	Revised footnote "r" to clarify that HCVRNA would be performed only if HCVAb is positive.	• To improve clarity.
Table 1 Schedule of Assessments	Added footnote "c" to indicate that Visit 9 MRI and liver biopsy procedures are required to be completed within +14 days of Visit 9.	• Ensures that Visit 9 liver biopsy and MRI are done after the last dose of study drug.
Table 1 Schedule of Assessments	Added footnote "d" to clarify that subjects who have failed screening may be re-screened.	• To allow the rescreening of subjects who previously screen failed, once their eligibility status has changed from the initial screening visit.
Section 3.1 Study Design and Flow Chart, Synopsis	Added mention that the number of F0 subjects will be capped at 81 if 1 dose is dropped after the interim analysis and at 62 if 2 doses are dropped.	• To allow for a limited number of subjects without fibrosis to be enrolled.
Section 3.1 Study Design and Flow Chart, Synopsis	Added mention that 208 subjects are needed for analysis if 1 or 2 doses of SHP626 are dropped after the interim analysis.	• To improve clarity.

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **3**	**Amendment Date** **22 March 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Section 4.1 Inclusion Criteria, Synopsis	Criterion #6 was revised to allow for F0 (fibrosis score), in addition to F1-F3.	• To allow for subjects without fibrosis to be eligible for enrollment.
Section 4.2 Exclusion Criteria, Synopsis	Revised criterion #5 to include: "Stents or other devices may be allowed, at the investigator's discretion, if they do not interfere with the functioning of the MRI machine."	• To allow for inclusion of subjects having these devices, at the discretion of the investigator.
Section 4.2 Exclusion Criteria, Synopsis	Criterion #8 was revised to update the definition of uncontrolled diabetes (HbA1c ≥9.5% within 60 days prior to enrollment).	• To allow for patients with up to 9.5% HbA1c to be eligible for participation.
Section 4.2 Exclusion Criteria, Synopsis	Revised criterion #9 to clarify that insulin dose may be adjusted after screening/enrollment as long as the subject's HbA1c remains <9.5%.	• To allow for inclusion of subjects who may need to adjust insulin dose during the study.
Section 4.2 Exclusion Criteria, Synopsis	Revised criteria #11 and #12 to allow for enrollment of subjects with elevation in AST (#11) and ALT (#12) up to 7 times the ULN (instead of 5 times the ULN).	• It was determined that elevations up to this point (≤7 times ULN) in AST and ALT should not preclude subject participation and does not expose such subjects to any additional safety risks.
Section 4.2 Exclusion Criteria, Synopsis	Revised criterion #30 to remove exclusion of subjects who previously failed screening.	• To allow the rescreening of subjects who previously screen failed, once their eligibility status has changed from the initial screening visit.
Section 4.2 Exclusion Criteria, Synopsis	Revised criterion #31 regarding exclusion of investigational study site employees.	• To improve clarity.
Section 5.2.2 Prohibited Treatment	Added obeticholic acid to the list of prohibited treatments.	• Prohibited this medication as there is potential for a drug to affect NASH.
Section 5.2.2 Prohibited Treatment	Clarified that tetracycline dose limit is ≤1 g per day.	• To improve clarity.
Section 5.2.2 Prohibited Treatment	Clarified that estrogen dose >2 mg QD is prohibited.	• To improve clarity.

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **3**	**Amendment Date** **22 March 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Section 5.2.2 Prohibited Treatment	Changed text from "drugs cannot have been taken for more than 2 weeks within the year prior to randomization" to: "drugs cannot have been taken for more than 2 weeks within the 6 months prior to randomization"	• To enable a less strict requirement without affecting safety.
Section 6.2.2 Allocation of Subjects to Treatment	Added mention that the number of F0 subjects will be capped at 81 if 1 dose is dropped after the interim analysis and at 62 if 2 doses are dropped.	• To allow for a limited number of subjects without fibrosis to be enrolled.
Section 6.2.3 Dosing	Added mention that if a dose is missed at the normally scheduled time, the subject can make up the dose that day as long as no more than 1 dose is taken in a 10-hour period.	• To specify parameters regarding dosing time if a dose is missed
Section 7.1.1 Screening (Visit 1)	Revised passage to indicate subjects designated as screen failures could be re-screened once.	• To allow the rescreening of subjects who previously screen failed when their eligibility status has changed from the initial screening visit.
Section 7.1.1 Screening (Visit 1)	Added description of process for providing additional liver tissue to the central reader.	• To allow additional liver tissue to be sent to the central reader.
Section 7.1.2 Baseline (Visit 2)	Added mention that subjects are randomized using IRT before the IA and then to 1 of the remaining arms after the IA.	• To improve clarity.
Section 7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)	Added details of the liver histology assessment at Week 48.	• To improve clarity.
Section 7.2.3.6 Additional Laboratory Assessments	Clarified that HCVRNA would be performed only if HCVAb is positive.	• To improve clarity.
Section 7.2.3.6 Additional Laboratory Assessments	Revised to clarify that Vitamin A, D, and E testing are not required at screening.	• To improve clarity. • Alignment with Table 1.

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **3**	**Amendment Date** **22 March 2017**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
Section 9.3 Data Handling and Considerations	Removed "IP serum concentrations, antibiotics to investigation product" from the parenthetical list of data items that could potentially unblind treatment assignement.	• To correct an error.
Section 9.6 Sample Size Calculation and Power Considerations, Synopsis	Increased approximate number of subjects screened from 334 to 677 subjects.	• To accommodate a higher screen failure rate.
Section 9.6 Sample Size Calculation and Power Considerations, Synopsis	Added specificity with regard to number of subjects needed for analysis if more than 1 dose/dosing arm is dropped following the interim analysis.	• To improve clarity.
Section 9.7 Study Population	Added definition of "The Randomized Set."	• To improve clarity.
Section 9.8 Efficacy Analyses	Clarified that the efficacy analyses involving statistical testing will only be done for the doses that are not dropped at the interim analysis.	• To improve clarity.
Section 9.8.1 Primary Efficacy Endpoint	Clarified that the method for handling missing data in the primary analysis is non-responder imputation.	• To improve clarity.
Section 9.8.2 Secondary Efficacy Endpoints	The definition of "Resolution of NASH" was revised.	• To allow consistency with newly revised and recommended definition.
Throughout protocol.	Minor changes to format and correct typos.	• To improve clarity.

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious Adverse Events (AEs) Required by the Protocol Form within 24 hours to:

Shire Global Drug Safety Department

Preferred method: scan and e-mail to globalpharmacovigilance@shire.com
OR fax to +44(0) 1256 89 4715 (Global); or +1 866 557 4473 (North America)

AND

A copy of this form must also be sent to the Shire GCDL by fax or e-mail using the details below:

Melissa Palmer, MD, FAASLD, GCDL-Hepatology

fax number: +1 781 482 1822

e-mail: mpalmer@shire.com

For protocol- or safety-related issues during normal business hours 9:00 a.m. to 5:00 p.m. (Eastern Time), the investigator must contact the Contract Research Organization (CRO) Medical Monitor:

ICON Medical Monitor: Anthony Japour, MD

Telephone number: +1 215 616 6439
Fax number: +1 215 616 3096
Mobile number: +1 267 429 6601
E-mail address: Anthony.Japour@iconplc.com

For protocol- or safety-related issues outside of normal business hours, the investigator must contact ICON's 24/7 Medical Emergency Coverage:

Chargeable global telephone number: +1 919 674 5468

NOTE: Investigative sites will be provided country-specific toll-free telephone numbers. Please refer to this document, as applicable. Countries without a toll-free number will need to dial the chargeable number noted above.

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

Please use the information below as applicable to report the Product Quality Complaint:

Origin of Product Quality Complaint	**E-mail Address**
North and South America	PQC@shire.com
European Union and Rest of World	PQCROW@shire.com

Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
1-888-300-6414 or 1-800-828-2088

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ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPPT	activated partial thromboplastin time
ASBT	apical sodium-dependent bile acid transporter
ASBTi	ASBT inhibitor
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BA	bile acid
BMI	body mass index
C4	7-alpha-hydroxy-4-cholesten-3-one
CRA	clinical research associate
CRC	clinical research center
CRO	contract research organization
DMC	data monitoring committee
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimension-5 Level Questionnaire
ECG	electrocardiogram
EOS	end of study
EU	European Union
FAS	full analysis set
FBA	fecal bile acid
FDA	Food and Drug Administration
FXR	farnesoid X receptor
GCDL	Global Clinical Development Lead
GCP	Good Clinical Practice
GDS	Global Drug Safety
GGT	gamma glutamyl transferase
GLP-1 RA	glucagon-like peptide-1 receptor agonists
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus

HBVDNA	hepatitis B virus deoxyribonucleic acid
HCV	hepatitis C virus
HCVAb	hepatitis C virus antibody
HCVRNA	hepatitis C virus ribonucleic acid
HDL-C	high-density lipoprotein-cholesterol
HFD	high fat diet
HFF	hepatic fat fraction
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HOMA-%B	homeostasis model assessment in β-cell function
HOMA-IR	homeostasis model assessment insulin resistance
HRQoL	health-related quality of life
IA	interim analysis
IAS	interim analysis set
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	investigational new drug
INR	international normalized ratio
IP	investigational product
IR	insulin resistance
IRB	Institutional Review Board
IRT	interactive response technology
LDL-C	low-density lipoprotein-cholesterol
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MOA	mechanism of action
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NOAEL	no observed adverse effect level
PBC	primary biliary cirrhosis

PBO	placebo
PDFF	proton density fat-fraction
PO	by mouth
POC	proof of concept
PRO	patient-reported outcome
PSC	primary sclerosing cholangitis
PT	prothrombin time
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAF	steatosis, activity, and fibrosis score
SAP	statistical analysis plan
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TA	therapeutic area
TB	total bilirubin
TEAE	treatment-emergent adverse event
TGR5	transmembrane G protein-coupled receptor
TPN	total parenteral nutrition
TSH	thyroid-stimulating hormone
TZD	thiazolidinediones
ULN	upper limit of normal
USA	United States of America
VAS	visual analogue scale

STUDY SYNOPSIS

Protocol number: SHP626-201	**Drug:** Volixibat potassium (SHP626)
Title of the study: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)	
Number of subjects (total and for each treatment arm): Approximately 677 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 placebo [PBO]) a 24-week interim analysis (IA) will eliminate at least one dose group based on the criteria provided to the Data Monitoring Committee; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue; 208 (accounts for 20% of dropouts) subjects are needed if two SHP626 doses are dropped at the IA.	
Investigator(s): Multicenter study	
Site(s) and Region(s): Anticipated regions -US, Canada, and EU Estimated number of sites planned – 60 to 80	
Study period (planned): July 2016 to July 2020	**Clinical phase:** 2

Objectives:

Primary: To evaluate the effect of volixibat compared to PBO on liver histology

Secondary:

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual nonalcoholic fatty liver disease (NAFLD) activity score (NAS) components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

Rationale: Currently, there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its mechanism of action (MOA) and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

Investigational product, dose, and mode of administration:

- Volixibat 5, 10, and 20 mg and matched PBO capsules by mouth (PO) once daily (QD). Investigational product (IP) should be given 30 minutes prior to the first meal of the day containing approximately 10-20

grams of fat. Also see Section 6.2.3 (Dosing).

- Identical PBO will be used as comparator
    - Subjects should take the IP at the same time each day and should not take more than one dose in a day if they miss a dose. If a dose is missed at the normally scheduled time, the subject can make up the dose that day as long as no more than 1 dose is taken in a 10-hour period.

Methodology:

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have received 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 81 if 1 dose is dropped after the interim analysis and at 62 if 2 doses are dropped. Depending on the results of the IA, the study may be terminated or the randomization to one or two doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

Inclusion and exclusion criteria:

Inclusion Criteria:

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally-authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and 30 days following the last dose of the IP.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F0-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

Exclusion Criteria:

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV [defined as HCVAb positive] without a current or prior history of a detectable HCVRNA) will be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the IP (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant

diarrhea, or history of chronic diarrhea or loose stools of any etiology.

4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver biopsy and/or MRI within 6 months of screening, but experienced a weight change ≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.
5. Contraindications to MRI (ie, claustrophobia, coronary stents, coronary implantable devices, girth, etc.). Stents or other devices may be allowed, at the investigator's discretion, if they do not interfere with the functioning of the MRI machine.
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation.
8. Uncontrolled diabetes defined as HbA1c of ≥9.5% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening, or a sliding scale of insulin is allowed as long as the subject's HbA1c remains < 9.5%.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to qualifying liver biopsy, or initiated any time after qualifying liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST >7 times upper limit of normal (ULN) at screening.
12. Serum ALT >7 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3.
15. Total bilirubin (TB) ≥2.0 x ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$L
17. Medical history of impaired hemostasis or current use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie, 81mg, aspirin (ASA) or clopidogrel (Plavix), will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's

opinion, may impact this Shire-sponsored study

23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.
25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior sponsor approval).
31. Subjects who are employees at the unit of the investigational site that is conducting the study.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 56 days
- Planned duration of enrollment period: 364 days
- Planned duration of treatment period: 336 days
- Planned duration of follow-up: 28 days

Endpoints and statistical analysis:

Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an adverse event (AE) or reporting the absence of AEs).

Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the time of the data cut for the IA.

Screened Set will consist of all subjects who have signed an informed consent.

Randomized Set will consist of all subjects in the Screened Set who have been randomized into the study.

Efficacy Endpoints:

- ***Primary:*** Binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS.
- ***Secondary:***
  - Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
  - Change from baseline to Week 48 on hepatic steatosis as measured by MRI-PDFF.
  - Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH Clinical Research Network (CRN))

- Resolution of NASH (defined as total absence of ballooning [score = 0] absent or mild inflammation [score 0-1], steatosis can be present [score 0-3]), without worsening of fibrosis as assessed by liver histology at week 48.
- Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
  - alanine aminotransferase (ALT)
  - aspartate aminotransferase (AST)
  - alkaline phosphatase (ALP)
  - gamma glutamyl transferase (GGT)
  - total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
  - fasting serum glucose levels
  - insulin levels
  - HbA1c
- Change from baseline to Week 48 on serum lipids measured by:
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

Primary hypotheses:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.
- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

The FAS will be used to assess the primary efficacy endpoint. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. Subjects with missing data for the primary efficacy endpoint at the Week 48 visit will be considered as non-responders. The test will be stratified by presence or absence of Type 2 diabetes mellitus (T2DM) at baseline, and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level. Histology will be read by one central hepatohistopathologist who will use the NASH CRN standard scoring system – NAS. The SAF Steatosis (S), Activity (A), and Fibrosis (F) scoring system will be determined for exploratory purposes.

The Safety Analysis Set will be used to assess the safety endpoints including AEs (including changes from baseline in physical examination findings), vital signs, ECGs, and clinical laboratory tests (chemistry, hematology, coagulation and urinalysis).

AEs will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as AEs that started or worsened on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be presented by system organ class, preferred term, and by treatment group. TEAEs will be further summarized by severity and the relationship to the IP. AEs related to the IP, AEs leading to withdrawal, SAEs, and death will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

For safety parameters, baseline is defined as the last assessment prior to the first dose of the IP.

Planned Interim Analysis:

An IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have

received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO.

Tolerability will be assessed by the number of discontinuations due to any one TEAE, presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Efficacy at the IA will be based on reduction of steatosis or ALT. Steatosis is assessed by MRI-PDFF. Depending on the results from the IA, one or more doses of volixibat may be discontinued or the study may be terminated. If the study is not terminated, subjects will receive a total of 48 weeks of the IP.

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9[c] EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Informed consent[d]	X									
Inclusion/exclusion criteria	X	X (review)								
Demography, medical & medication history	X									
Physical examination	X	X					X		X	X
Height[e], weight[f], waist circumference and waist:hip ratio	X	X	X	X	X	X	X	X	X	X
Vital signs[g]	X	X	X	X	X	X	X	X	X	X
PRO (EQ-5D-5L)		X					X		X	
Liver biopsy[h]	X								X	
MRI[i]	X						X[j]		X	
ECG (12-lead)	X	X				X			X	
Biochemistry and Hematology[k]	X	X	X	X	X	X	X	X	X	X
Serum Glucose[l]	X	X	X	X	X	X	X	X	X	X
Urinalysis[m]	X	X							X	
Urine Drug and Blood Alcohol Tests	X	X								
Urine Pregnancy Test[n]	X	X	X	X	X	X	X	X	X	X
Lipid Panel[0]	X	X	X	X	X	X	X	X	X	X
Coagulation Panel[p]	X	X		X			X		X	

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9[c] EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Vitamins A, D, and E[t]		X		X			X		X	
HbA1c[k]	X	X	X	X	X	X	X	X	X	X
Serum Liver-Related Blood Tests[k, q]	X	X	X	X	X	X	X	X	X	X
Insulin[k]	X	X		X		X	X	X	X	X
HIV, Hepatitis B/C[k, r]	X									
Thyroid testing[k, s]	X	X		X		X	X		X	X
C4 Sampling		X				X	X		X	X
IRT Accessed	X	X	X	X	X	X	X	X	X	X
Randomization		X								
IP Dispensed[t]		X	X	X	X	X	X	X		
IP Returned/Accountability & Compliance Assessed			X	X	X	X	X	X	X	
Stool Assessment[u]		X	X			X		X	X	X
Adverse Events[v]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X

ECG=electrocardiogram; EOS=end of study; HOMA=Homeostasis Model Assessment; IA=interim analysis; IP=investigational product; IRT=Interactive Response Technology; MRI=magnetic resonance imaging; PRO=patient-reported outcome.

[a] Visit Windows (calculated from Visit 2): Bi-weekly (Visits 3-4): +/- 3 days; Monthly (Visits 5-6, Visit 10): +/-5 days; Tri-monthly (Visits 7-9/DC): +/- 7 days.

[b] Subjects will be reminded not to eat prior to their scheduled visit. Additionally, during the double-blind treatment period, they should not take their study drug prior to the visit. They should bring their study drug with them to the visit to take 30 minutes prior to their first meal of the day containing approximately 10-20 grams of fat. Also see Section 6.2.3

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9[c] EOS	10 In-Clinic
Week	-8 to 0	0	2	4	8	12	24	36	48	52
Study Day	-56 to -1	0	14	28	56	84	168	252	336	364

[c] Subjects are to complete the Visit 9 liver biopsy and MRI procedures within +14 days of Visit 9. All other Visit 9 assessments are to be completed within a window of +/- 7 days.

[d] Subjects who completed screening and were designated as a screen failure may be rescreened. However, rescreened subjects must begin the screening procedure again, must be re-consented and will be assigned a new subject number.

[e] Height to be measured at screening only.

[f] BMI to be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7 and 9/DC.

[g] Vital signs to include oral, temporal, or tympanic temperature, sitting blood pressure, pulse, and respiratory rate.

[h] Biopsy performed within 6 months of screening can be used. All biopsies will be centrally read by a hepatic histopathologist. See Sections 7.1.1 and 7.1.3 for additional details.

[i] MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.

[j] MRI at Visit 7 (Week 24) for the Interim Analysis Set only.

[k] All blood tests are fasting blood tests. Subjects are not required to have Vitamin A, D, and E testing at screening (Visit 1).

[l] HOMA-IR and HOMA-%B: will be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7, 9/DC, and 10.

[m] Urinalysis to include oxalate testing.

[n] For all females of child-bearing potential (FOCP). Positive on-site urine dipstick results must have serum β-HCG testing performed by central lab.
Additional testing can be performed at the investigator's discretion.

[o] Lipid Panel includes fasting total cholesterol, HDL-C, LDL-C, and triglycerides.

[p] Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points to assess vitamin K level.

[q] Serum Liver-Related Blood Tests include ALT, AST, ALP, GGT, and total bilirubin.

[r] Hepatitis B/C testing includes HBcAb, HBsAg, HBVDNA and HCVAb (if HCVAb is positive an HCVRNA will be performed), respectively.

[s] Thyroid testing includes thyroid stimulating hormone (TSH) and triiodothyronine (T3).

[t] Investigational product may be dispensed at an unscheduled visit outside of this schedule as needed to replace lost or damaged product.

[u] Subjects will be queried about the number of stool evacuations during the 24- hour period before the clinical research center (CRC) visit and asked to describe the consistency of the softest stool during that 24- hour period using the Bristol Stool Chart.

Table 1 Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9[c] EOS	10 In-Clinic
Week	-8 to 0	0	2	4	8	12	24	36	48	52
Study Day	-56 to -1	0	14	28	56	84	168	252	336	364

[v] Adverse events will be collected beginning from the signing of informed consent. All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

1. BACKGROUND INFORMATION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide (Vernon et al. 2011), and is estimated to occur in 30-40% of adults in the United States and up to 30% of European adults. These numbers approach 95% in those with morbid obesity (Mathus-Vliegen et al. 2012). NAFLD ranges from simple steatosis, which is typically nonprogressive, to nonalcoholic steatohepatitis (NASH), which has a 20% likelihood of progression to advanced disease including fibrosis, cirrhosis and its complications including liver failure and the need for a liver transplant (Angulo 2002). Hepatocellular carcinoma is also a complication of NASH that may occur with or without the presence of cirrhosis (Torres et al. 2012). NAFLD is typically associated with type 2 diabetes mellitus (T2DM) (Adibi et al. 2007; Loomba et al. 2012), central or visceral obesity (Souza et al. 2012), dyslipidemia (Assy et al. 2000; National Cholesterol Education Program 2002), and hypertension (Donati et al. 2004). Together these conditions comprise the metabolic syndrome, and NASH is considered to be the hepatic component of this syndrome (Hamaguchi et al. 2005; Neuschwander-Tetri 2005; Marchesini et al. 2005). Notably, NAFLD is a clinical condition occurring in individuals who do not drink excessive alcohol (>20 grams/day), yet have hepatic histology which is indistinguishable from that seen with alcoholic excess. The pathophysiology of NASH is likely multifactorial and may include combinations of metabolic, genetic, environmental, and gut microbial factors.

Most individuals with NASH are asymptomatic or have nonspecific symptoms such as fatigue. They typically first come to medical attention incidentally following routine blood testing or on imaging studies performed routinely or during the evaluation of an unrelated condition. While ultrasound and magnetic resonance imaging (MRI) can detect the presence of steatosis (Reeder et al. 2011), a liver biopsy is required to diagnose NASH and the extent of liver fibrosis.

1.1 Indication and Current Treatment Options

There are currently no drugs approved for the treatment of NASH and it is estimated that there are between 6-16 million people in the United States with NASH, of which 600,000 have severe disease (Williams et al. 2011 and Torres and Harrison 2008), with similar percentages reported throughout most areas of the world (World Gastroenterology Organisation 2012). Treatment of associated metabolic comorbidities, weight reduction, and incorporation of an exercise routine remain the cornerstone of management. However, lifestyle changes are seldom successful. Thus, NASH represents a disease with an unmet medical need that is growing at an epidemic rate, and that if untreated, carries a risk of significant morbidity and mortality.

1.2 Product Background and Clinical Information

Volixibat potassium (SHP626; formerly LUM002), hereafter referred to as volixibat, is a highly selective inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that is being evaluated for the treatment of NASH.

Bile acids (BA) promote bile flow, activation of digestive enzymes, and micellization of fats and fat-soluble vitamins, thereby permitting their intestinal absorption. BAs serve as signaling molecules acting via receptors, such as farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR5), in the intestine, liver and other tissues which play an important role in regulating insulin homeostasis. (Halilbasic et al. 2013). Lipid peroxidation and oxidant stress have been proposed as one link between the accumulation of fat and subsequent injury (Day and James 1998). All of these metabolic actions have important effects to prevent the ongoing liver damage in NASH.

High fat diet (HFD) fed mice treated with an ASBT inhibitor (ASBTi) (SC-435 a surrogate SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved insulin resistance (IR), and decreased NAS, predominantly steatosis. In addition the BA pool reflected increases in BA that were agonists to FXR and decreases in levels that were antagonist to the FXR (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015). Miethke and colleagues (2016) found that pharmacological inhibition of the ileal, ASBT (SC-435 the surrogate molecule of volixibat), blocked progression of sclerosing cholangitis in mdr2–/– mice. Beneficial effects in liver histology in these mice included a reduction in the severity of hepatic fibrosis, a decrease which correlated with a reduction of hepatic profibrogenic gene expression. While very encouraging, whether or not the findings in this mouse sclerosing cholangitis model can translate to histologic improvements in patients with NASH is unknown.

Volixibat, as a potent inhibitor of ASBT, increases BA excretion and facilitates signaling in the intestine that regulates serum and hepatic BA concentrations, glucose metabolism, serum cholesterol and fatty acid metabolism in the liver. The combined events resulting from inhibiting BA reuptake are hypothesized to have a positive metabolic, anti-inflammatory, anti-steatotic, and potentially antifibrotic effect that will lead to a therapeutic benefit for patients with NASH.

One of the factors contributing to the pathogenesis of NASH is abnormal cholesterol metabolism and the accumulation of free cholesterol in the liver. The free cholesterol is directly toxic to hepatocytes, which leads to inflammation and fibrosis (Musso et al. 2013). One treatment approach is to remove the cholesterol from the liver to decrease and possibly reverse the damage to the hepatocytes. This is one of the mechanisms by which SHP626 will treat NASH.

Due to the mechanism of action (MOA), volixibat is under development for the treatment of NASH and may also be able to improve the metabolic syndrome that is associated with NASH. Volixibat inhibits ASBT therefore BAs are excreted in the feces and this loss forces the liver to synthesize new BA which utilizes cholesterol in the liver and serum. Volixibat also has the potential to reduce IR, which is considered to be the most common underlying risk factor for the development of NASH (Pagano et al. 2002; Sanyal et al. 2001).

Volixibat was initially being evaluated for its use as an intervention for dyslipidemia. As a result of initial observations in animals that serum low-density lipoprotein cholesterol (LDL-C) and total liver cholesterol content could be decreased after administration of volixibat, additional work was undertaken to support the safety and tolerability of the drug in healthy volunteers. Clinical and non-clinical studies have demonstrated very low systemic exposure across species.

Oral administration of volixibat at doses up to 300 mg once daily and 50 mg administered daily for 14 days to healthy male subjects was generally safe. A Phase 1 study revealed that volixibat is tolerated at the 10 mg dose for 28 days and that there were trends towards increasing high-density lipoprotein cholesterol (HDL-C), decreasing LDL-C, and decreasing fasting glucose in patients with T2DM (LUM002-101 trial).

A healthy volunteer study investigating 12 days of varying levels of repeat doses of volixibat has provided key pharmacodynamic information for dose selection as measured by the effect of volixibat on excreted fecal bile acid (FBA) levels (SHP626-101). In addition, the study investigated safety and tolerability over the dose range that was considered for this Phase 2 study.

Data from Phase 1 studies support the position that volixibat is basically a non-absorbed drug that works locally in the GI tract and results in virtually no systemic exposure. Thus pharmacokinetic sampling will not be done in this study.

The current proof of concept (POC) Phase 2 trial will evaluate the safety, tolerability, and efficacy of three doses of volixibat (5, 10, and 20 mg) in adult subjects with NASH. Due to the MOA of volixibat, efficacy will be assessed at an interim analysis (IA) by a change of steatosis from baseline to Week 24 compared to placebo (PBO). While the gold standard for quantification of steatosis has historically been an invasive liver biopsy, quantitative magnetic resonance (MR) imaging–based biomarkers for liver fat have evolved rapidly over the last decade, and are increasingly being incorporated into NASH clinical trials. Both MR spectroscopy (S) and MR imaging (I) proton density fat-fraction (PDFF) provide non-invasive means of quantifying intrahepatic lipid content (Reeder et al. 2012). Both techniques have been shown to be accurate, reproducible with a low degree of variability in interpretation, cost-effective and reliable biomarkers of quantitative hepatic fat (Roldan-Valadez et al. 2010; Urdzik et al. 2012; Raptis et al. 2012). Importantly, studies demonstrate a close correlation with steatosis grade histologically (Qayyum et al. 2005; Schwenzer et al. 2009).

In a study of 51 adult subjects with NAFLD, PDFF correlated well with the grade of histologic steatosis, as the mean fat-fraction values of 8.9%, 16.3%, and 25.0% corresponded to histologic steatosis grades 1, 2, and 3, respectively ($P < .0001$) (Permutt et al. 2012). Thus, MRI will be utilized to evaluate the degree of steatosis change from baseline in this study during the IA. The Mozart trial was a randomized, double-blind, PBO-controlled trial of 50 patients with NASH who were randomized to 24 weeks of either ezetimibe or PBO, evaluating the reduction of liver fat by MRI-PDFF as well as by histology. Results revealed that compared to histologic non-responders, histologic responders, defined as a two-point reduction in NAFLD activity score (NAS) without worsening fibrosis, had a statistically significant reduction in net MRI-PDFF of $-4.1\% \pm 4.9$ vs. $+0.6\% \pm 4.1$ ($P <0.036$) with a mean percent change of $-29.3\% \pm 33.0$ vs. $+2.0\% \pm 24.0$ ($P < 0.004$), respectively (Loomba et al. 2015). Thus, in the current trial during the IA, a $\geq$5% steatosis reduction for an active dose compared to PBO will be a clinically meaningful change after 24 weeks of therapy.

Intrahepatic lipid content of less than 1% is considered to be within the normal range (Springer et al. 2015), however, from a study of 2349 people in a general population undergoing MRS, it was concluded that a PDFF value of 5.56% represented the upper limit of the normal range, as determined from the 95th percentile of PDFF in 345 individuals who were not at increased risk for hepatic steatosis (Szczepaniak et al. 2005). Thus, in the current trial, similar to other NASH trials utilizing MR for evaluation, an MRI ≥5% steatosis will be used as an inclusion criterion (Loomba et al. 2015).

1.3 Benefits and Risks

By virtue of volixibat's ability to inhibit ASBT bile acid reabsorption, there is an increase in BA excretion and signaling in the intestine that results in improvements in glucose metabolism and changes in cholesterol and fatty acid synthesis in the liver. Recently, HFD fed mice treated with an ASBTi (SC-435 a surrogate of SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved IR, and decreased NAS (predominantly steatosis). In addition, these HFD-fed mice did not gain weight when treated with SC-435, in spite of consuming increased calories. Finally, the BA pool in these mice changed to predominantly FXR agonist (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015).

These metabolic actions and preclinical results may prove to be clinically relevant to subjects with NASH.

NASH has recently received considerable attention as awareness of the problem of liver damage and prevalence of the disorder has increased, paralleling the obesity epidemic. Consequences of liver damage are detrimental and can lead to liver failure, hepatocellular carcinoma, and the need for liver transplantation. There is no currently approved medical therapy for NASH. The large unmet medical need and the increased medical resource burden have led to the search for potential therapies to treat NASH.

Nonclinical testing established that the no observed adverse effect level (NOAEL) for volixibat in rats and dogs following 13 weeks of once-daily administration were 1000 and 500 mg/kg/day, respectively. Similarly, testing confirmed that the NOAEL for volixibat in a 6-month study in rats and a 9-month study in dogs were 1000 and 500 mg/kg/day, respectively. In both cases, these were the highest doses tested. Genotoxicity testing has yielded negative findings.

Volixibat is minimally absorbed. The pharmacokinetic profiles performed in clinical studies completed to date repeatedly suggest negligible systemic exposure. Furthermore, there has been no observation of clinically relevant changes in fat absorption parameters such as those related to fat-soluble vitamins.

The most frequent TEAEs in the Phase 1 studies were GI and were considered mechanism-based due to elevated BA concentrations in the colon. The percentage of subjects reporting at least 1 TEAE in the GI disorders SOC generally increased with an increasing volixibat dose level (Part 1 Study TDU10632 and Study LUM002-101). Most TEAEs were mild in intensity, and none were assessed as severe.

In the multiple dose studies, the most commonly reported TEAEs in subjects (both healthy and with T2DM) who received volixibat for the longest duration of 28 days in Study LUM002-101 included diarrhea and abdominal pain. The most commonly reported TEAEs in subjects receiving 50 mg volixibat for 14 days (part 3 Study TDR10633) were diarrhea and GI pain.

Overall, there were 2 SAEs (ALT increased and retinal detachment), both of which led to the discontinuation of volixibat. In part 3 of the initial Phase 1 study (TDU10633), 1 subject dosed with 50 mg volixibat for 13 days was withdrawn from the study due to a mild TEAE (which became a SAE due to prolonged hospitalization) of ALT increased that was considered related to volixibat . The subject's ALT level returned to normal after discontinuation of volixibat. A second subject dosed with 10 mg volixibat for 12 days in Study LUM002-101 reported a moderate SAE of ablation of the retina with a bleed in the vitreous body of the right eye that was considered not related to volixibat.

Overall, 3 subjects, all dosed with 5 mg volixibat in Study LUM002-101, discontinued volixibat due to non-serious TEAEs: 1 due to a related TEAE of mild hemorrhagic diarrhea, 1 due to an unrelated TEAE of moderate Epstein-Barr virus infection, and 1 due to mild related TEAEs of diarrhea and anal erosion.

Overall, the observed AEs attributable to volixibat have been self-limited as would be expected given the local MOA of ASBT inhibition in the terminal ileum. Generally, among subjects who experienced GI TEAEs, the events have been mild and diminished over the course of treatment. Please refer to the investigator's brochure for additional information.

Volixibat is a novel drug candidate, demonstrating limited systemic exposure across species with the potential to affect important metabolic pathways associated with NASH. The overall safety, tolerability, and preliminary activity of volixibat in available clinical trials suggest that further investigation is warranted and that there is a positive benefit to risk profile.

Always refer to the latest version of the Volixibat Potassium (SHP626) Investigator's Brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of volixibat.

See Appendix 1 for protocol history, including all amendments.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Currently there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its MOA and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

2.2 Study Objectives

2.2.1 Primary Objective

To evaluate the effect of volixibat compared to PBO on liver histology

2.2.2 Secondary Objectives

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual NAS components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

2.2.3 Exploratory Objectives

- To explore the effect of volixibat compared to PBO on liver histology (measured by individual SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F))
- To explore the effect of volixibat compared to PBO on anthropometric measures (body weight, body mass index (BMI), waist circumference and waist-hip ratio)
- To explore the effect of volixibat compared to PBO on homeostasis model assessment-IR (HOMA-IR) and HOMA-beta cell function (HOMA-%B) in subjects with T2DM
- To explore the effect of volixibat compared to PBO on BA synthesis (7-alpha-hydroxy-4-cholesten-3-one [C4])
- To explore the effect of volixibat on patient-reported health-related quality of life (HRQoL) and overall health status.

3. STUDY DESIGN

3.1 Study Design and Flow Chart

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio such that a target of 266 subjects is achieved (221x1.2 is

approximately 266). The 221 subjects include 201 subjects for the three arms analyzed at 48 weeks plus an additional 20 subjects to account for one dose dropped at the IA. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue; 208 (accounts for 20% of dropouts) subjects are needed if 2 SHP626 doses are dropped at the IA. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 81 if 1 dose is dropped after the IA and at 62 if 2 doses are dropped after the IA. Attempt will be made to perform the IA before any subject has had their 48-week post-treatment liver biopsy, although this will be dependent upon the rate of enrollment and dropout.

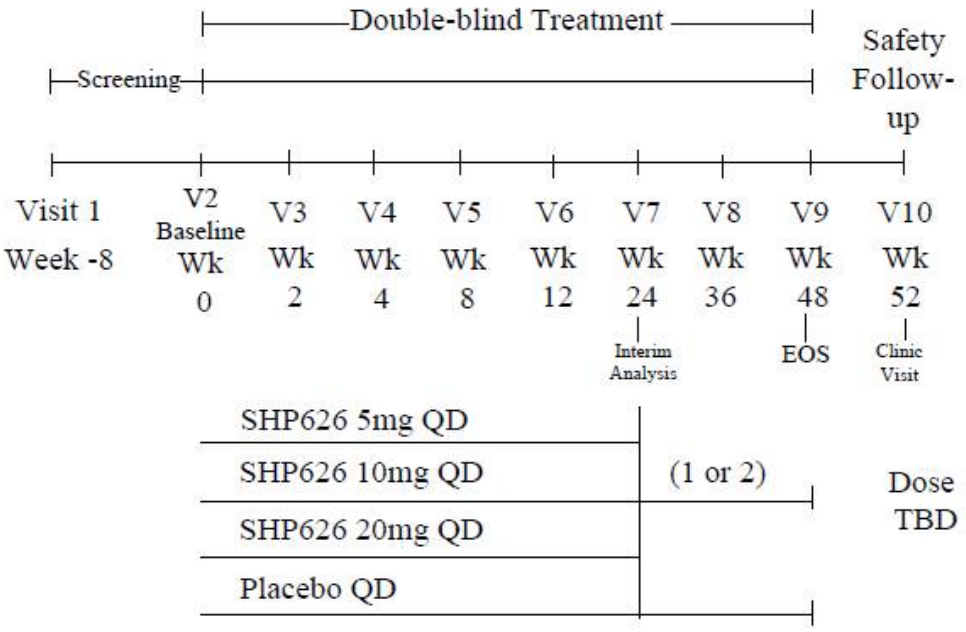
There will be up to 3 periods (screening, treatment, and follow-up) and an IA. The duration of the Treatment period will be 48 weeks, with an IA at Week 24. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

The IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. Study enrollment will be paused after 92 subjects have been randomized. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO.

Once decisions as to dose elimination are made, study enrollment will be reopened and additional sites may be added. Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining dose groups (noted by "[1 or 2]… Dose TBD" in Figure 1) or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of investigational product (IP).

The study will be conducted over 3 periods: screening (8 weeks), treatment (48 weeks), and follow-up (4 weeks), with an IA at 24 weeks as outlined in Figure 1.

Figure 1 Study Design Flow Chart



3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 364 days. The last visit is an in-clinic safety follow-up visit. The study will be completed in approximately 4 years.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at approximately 60 to 80 clinical sites in the USA, Canada, and EU.

4. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and for 30 days following the last dose of the IP as described in Section 4.4.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F0-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met.

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] positive and/or HBVDNA positive; subjects who are hepatitis B core antibody [HBcAb] positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV [defined as HCVAb positive] without a current or prior history of a detectable HCVRNA) may be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the investigational product (IP) (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver biopsy and/or MRI within 6 months of screening, but experienced a weight change of

≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.

5. Contraindications to MRI (eg, claustrophobia, coronary stents, coronary implantable devices, girth, etc.). Stents or other devices may be allowed, at the investigator's discretion, if they do not interfere with the functioning of the MRI machine.
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation (refer to Section 5.2.1, Permitted Treatment).
8. Uncontrolled diabetes defined as HbA1c of ≥9.5% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening, or a sliding scale of insulin is allowed as long as the subject's HbA1c remains < 9.5%. Also refer to Section 5.2.2, Prohibited Treatment.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to liver biopsy, or initiated any time after liver biopsy performed, including the use of total parenteral nutrition (TPN). Also refer to Section 5.2.2, Prohibited Treatment.
11. Serum AST >7 times upper limit of normal (ULN) at screening.
12. Serum ALT >7 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3
15. TB ≥2.0 x ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$/L
17. Medical history of impaired hemostasis or use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie 81 mg, aspirin (ASA) or clopidogrel (Plavix) will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.

21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Refer to Section 7.2.3.8, Drug and Alcohol screening. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.
25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior sponsor approval).
31. Subjects who are employees at the unit of the investigational site that is conducting the study.

4.3 Restrictions

Subjects must adhere to the following restrictions for the duration of the study:

- Subjects must remain compliant with inclusion/exclusion criteria.
- Subjects should not become pregnant, father a child, or nurse/breastfeed a baby.
- Subjects should be encouraged to adhere to the same exercise routine and a healthy diet throughout the study.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of child-bearing potential should use an acceptable form of contraception. Females of child-bearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of the IP. If hormonal contraceptives are used they should be administered according to the package insert.

Female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and age ≥51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of child-bearing potential with a negative urine and/or serum β-human chorionic gonadotropin (β-HCG) pregnancy test each study visit. Females of child-bearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of highly-effective contraception.

Acceptable methods of highly-effective contraception (ie, methods that result in a failure rate of <1% per year) are:

- Combined (estrogen and progestogen containing) hormonal contraceptives associated with inhibition of ovulation (oral, intravaginal, transdermal) stabilized for at least 30 days prior to the screening visit (Visit 1) plus a barrier method (eg, condoms or diaphragms with spermicidal gel or foam)
- Progestogen-only hormonal contraception associated with inhibition of ovulation plus a barrier method
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Male sterilization/vasectomized partner
- True abstinence: When this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.)

4.4.2 Male Contraception

Contraception is required for all sexually-active male subjects and their partners. All male subjects (including those who are sterile) agree not to donate sperm, and to use 1 of the following approved methods of contraception from the baseline visit on Day 0 until 30 days following study discharge:

- Male condom with spermicide
- Sterile sexual partner
- Intrauterine device with spermicide (use by female sexual partner)
- Female condom with spermicide (use by female sexual partner)
- Contraceptive sponge with spermicide (use by female sexual partner)
- Intravaginal system (eg, vaginal ring with spermicide, a diaphragm with spermicide, or a cervical cap with spermicide) (use by female sexual partner)
- Oral, implantable, transdermal, or injectable hormonal contraceptive (use by female sexual partner) plus a barrier method

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from the IP with the CRO Medical Monitor when possible.

If the IP is discontinued, regardless of the reason, the evaluations listed for both Visit 9/end of study (EOS) and Visit 10/Follow-up, are to be performed as completely as possible; however, the EOS liver biopsy will not be required for patients discontinuing prior to Week 44.

Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination, date of stopping the IP, and the total amount of the IP taken must be recorded in the electronic case report form (eCRF) and source documents.

Subjects who discontinue will not be replaced.

4.5.1 Subject Withdrawal Criteria

Medically important events that in the opinion of the investigator, medical monitor or sponsor would compromise the subject's ability to safely continue in the study may result in withdrawal of the subject from the study. Subjects who become pregnant or demonstrate disease progression, defined as the development of signs or symptoms of hepatic decompensation (ie,

esophageal variceal hemorrhage, hepatic encephalopathy, ascites, or hepatocellular carcinoma), will be withdrawn from the study and followed as set forth in the protocol.

4.5.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the eCRF. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event (AE)
- Protocol violation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Screen failure
- Noncompliance with study drug
- Physician decision
- Pregnancy
- Progressive disease (refer to Section 4.5.1)
- Study terminated by sponsor
- Other - If "Other" is selected, the investigator must specify on the eCRF

4.5.3 Subjects Lost to Follow-up Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact clinic visit. At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

4.5.4 Safety-related Stopping Rules

Refer to Appendix 4 and Appendix 5 for criteria to assess severity of liver-related AEs and stopping rules for liver-related blood tests, respectively.

An urgent safety review will be conducted within 7 days by the sponsor and in consultation with the DMC if 1 or more of the following criteria are met:

- Death that is considered related to the study drug
- Two SAEs of similar type (defined as same or similar Medical Dictionary for Regulatory Activities (MedDRA) higher level group code), and considered related to the study drug.

The urgent review will be performed by a sponsor safety review group, which will include the study Global Safety Lead (GSL) and the Global Drug Safety (GDS) therapeutic area (TA) Head, and in consultation with the DMC. The GDS TA Head, not the GSL involved in the study, may be unblinded as part of this urgent safety review, if required. Following the sponsor's review of safety data and consultation with the DMC, one of the following actions will be taken with respect to study status:

- Continue study with protocol unchanged
- Continue study with modifications to the protocol
- Terminate study

Subject safety will be monitored on a continuous basis during this study until the last subject completes his or her last scheduled study visit/assessment.

5. PRIOR AND CONCOMITANT TREATMENT

All non-study treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to signing informed consent at the screening visit (Visit 1) and through the final study contact (including protocol-defined follow-up period) must be recorded on the appropriate eCRF page.

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, and behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate), received within 30 days of the date of first dose of the IP. Prior treatment information must be recorded on the appropriate eCRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of the IP and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate eCRF page.

5.2.1 Permitted Treatment

Medications and supplements including but not limited to vitamin E, betaine, s-adenosyl-l-methionine, ursodeoxycholic acid, milk thistle, gemfibrozil, anti-TNF therapies, probiotics biguanides (metformin), thiazolidinediones (TZDs) and GLP-1 RAs that have been used to treat NAFLD/NASH are allowed during the course of the study if the subject has been on a stable dosing regimen (ie, same dose and frequency in the previous 6 months prior to the qualifying liver biopsy and not initiated after qualifying liver biopsy) and will continue this dosing regimen throughout study participation. Use of antiplatelet medications is also allowed. The investigator must contact the CRO medical monitor then the Shire GCDL to discuss any changes to concomitant medications that may impact the study.

5.2.2 Prohibited Treatment

The following list of prohibited drugs cannot have been taken for more than 2 weeks within the 6 months prior to randomization and are excluded while on study.

- Systemic Glucocorticoids
- Tamoxifen
- Amiodarone
- Methotrexate
- Alcohol (see Section 4.2)
- Griseofulvin
- Total parenteral nutrition
- Obeticholic acid
- Valproate
- Nucleoside Analogues (except acyclovir)
- Tetracycline (high dose; >1g)
- Estrogens at doses greater than 2 mg QD used for hormone replacement
- Anabolic steroids
- Bile acid sequestrants such as cholestyramine or colestipol
- Any other known hepatotoxins including over-the counter therapies and herbal therapies such as germander, chaparral and ma-huang.

This is not a comprehensive list. Treatments not listed above are generally considered allowable, unless considered a potential hepatotoxin. Antidiarrheals will be allowed at the discretion of the investigator, with the exception of BA sequestrants.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is volixibat potassium (volixibat), which will be provided in 5, 10 and 20 mg capsule form. Additional information is provided in the current Volixibat Potassium (SHP626) Investigator's Brochure.

The reference/comparator product is an identical PBO which will be provided in capsule form.

6.1.1 Blinding the Treatment Assignment

The IP will be supplied as double-blind blister packs. The actual double-blind treatment given to individual subjects is determined by a randomization schedule which will be automatically assigned by the interactive response technology (IRT). Placebo capsules, which exactly match the IP, will be used in the blister packs to provide the same number and size capsules for each of the doses within the treatment groups.

6.2 Administration of Investigational Product(s)

All IP and supplies will be provided by Shire or its designee**.** At each visit, subjects will be supplied with enough IP to last until the subsequent visit. Lost or damaged IP will be replaced as needed. Volixibat will be supplied to the clinical research center (CRC) as powder in capsule. Volixibat will be supplied in identical capsules in strengths of 5, 10, and 20 mg (with matched PBO).

6.2.1 Interactive Response Technology for Investigational Product Management

IRT will be used for the following investigational tasks:

- Randomization
- Supply management
- Inventory management and supply ordering
- Expiration tracking
- Returns
- Emergency unblinding

6.2.2 Allocation of Subjects to Treatment

This is a double-blind, PBO-controlled study. The actual treatment given to individual subjects is determined by a randomization schedule.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation.

The randomization number represents a unique number corresponding to the IP allocated to the subject, once eligibility has been determined.

Individual subject treatment is automatically assigned by the IRT.

Once a randomization number/unique identifier has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. If a randomization number/unique identifier is allocated incorrectly, the clinical research associate (CRA)/study monitor must be notified as soon as the error is discovered.

IP packaging identification numbers, separate from randomization numbers/unique identifiers, may also be assigned to subjects for specific treatment assignment as dictated by the study. In these cases, the same IP packing identification number may not be assigned to more than 1 subject.

Subjects will be equally allocated to all volixibat doses and PBO if their randomization precedes the 24 week IA. If a subject's randomization occurs after the 24 week IA, subjects will be equally allocated to all remaining volixibat doses (determined based on the results of the IA) and PBO. The randomization will be stratified by baseline T2DM and NAS $\geq$6 or NAS = {4,5}. Subjects with fibrosis stages F0 through F3 may be enrolled, but the number of F0 subjects will be capped at 81 if 1 dose is dropped after the interim analysis and at 62 if 2 doses are dropped.

6.2.3 Dosing

All doses of volixibat or matching PBO will be administered orally as a capsule in a double-blinded fashion. The first dose of IP for each subject will be administered in the clinic. The dose will be administered with 240 mL of water and should be given 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat. All assessments should be completed at least 30 minutes prior to administration of the IP. The subject should make all attempts to consistently take the IP around the same time each day. If a dose is missed at the normally scheduled time, the subject can make up the dose that day as long as no more than 1 dose is taken in a 10-hour period.

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be broken during the study except in emergency situations where the identification of the IP is required for further treatment of the subject. The investigator should contact the CRO medical monitor and the Shire GCDL at the same time and as soon as possible after the investigator has been unblinded.

In the event that the treatment assignment is broken, the date and the signature of the person who broke the code are to be recorded in the source documents and the IRT, and the reason for

breaking the code will be recorded in the source documents and the clinical database. Upon breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code-breaks that occur must be reported to the CRO medical monitor. Code-break information is held by the pharmacist/designated person at the site and by the CRO medical monitor for the study or designee.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled with a minimum of the protocol number, medication identification number, dosage form (including product name and quantity in pack), directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements 'For clinical trial use only', and/or 'CAUTION: New Drug - Limited by Federal (or USA) Law to Investigational Use', 'Keep out of reach of children', and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Additional labels may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The sponsor or designee will provide the IP for this study. The IP is packaged in the following labeled containers:

- Volixibat 5 mg capsules
- Volixibat 10 mg capsules
- Volixibat 20 mg capsules
- Volixibat PBO capsules

6.3.3 Storage

The investigator has overall responsibility for ensuring that the IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented.

IPs are distributed by the pharmacy or nominated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP labels as they are distributed.

All IP must be stored at the clinic at 20 - 25°C (68 - 77°F); excursions are allowed between 15 - 30°C (59 - 86°F).

IPs must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the IP is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion below 15°C (59°F) or above 30°C (86°F); these excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room or a change in storage location.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing the IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study

following the procedures set out in the study protocol. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed IP will be documented on the eCRFs and/or other IP record. The investigator is responsible to ensure the retrieval of all study supplies from subjects.

No IP stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records. The site must ensure that the accountability and destruction records are complete, accurate, and ready for verification at each monitoring visit.

The process for return and destruction of IP must be determined and documented during the study start-up phase.

With the written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject returned IP, and empty/used IP packaging may be destroyed at the site or a local facility on an ongoing basis throughout the study and at the end of the study following verification of accountability by the CRA/study monitor. In this case, destruction records identifying what was destroyed, when, how, and by whom, must be obtained with copies provided to the sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Alternatively, in the absence of written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject-returned IP, and empty/used IP packaging may be required to be sent to a nominated contractor on behalf of the sponsor for IP destruction on an ongoing basis throughout the study and at the end of the study. IP being returned to the sponsor's designated contractors also must be counted and verified by clinical site personnel and the CRA/study monitor. For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, IRT) do not require a shipment form. Returned IP must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any IP prior to shipment. Shipment of all returned IP must comply with local, state, and national laws.

6.5 Subject Compliance

Subjects must be instructed to bring their unused IP and empty/used IP packaging to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, blister pack) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details on the drug accountability form.

7. STUDY PROCEDURES

7.1 Study Schedule

This study will consist of a screening period of up to 56 days (the screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor), a 48-week Treatment period, and a Follow-up visit 4 weeks after treatment ends. A detailed display of all study procedures is provided in Table 1.

Patients will be assessed according to the following schedule:

- Screening – Visit 1 (Weeks -8 to 0 [Day -56 to Day -1])
- Baseline – Visit 2 (Week 0 [Day 0])
- Treatment and Assessments – Visits 3 through 9 (Week 2 through Week 48)
- Follow-up – Visit 10 (Week 52, 4 weeks after completion of dosing)

7.1.1 Screening (Visit 1)

Screening procedures must be completed within 56 days prior to randomization for the first dose of the IP. The screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor. At the screening visit, considered Visit 1 (Week -8 to 0, Day -56 to -1), all screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from the subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

The following screening procedures should be assessed at the beginning of the visit window, preferably between Day -56 and Day -49: inclusion/exclusion criteria, collection of subject information (demographics, medical and medication history AEs, and concomitant medications), a physical examination including vital signs, height, weight, and waist/hip measurements; collection of blood and urine samples for screening and safety assessments, performance of an electrocardiogram (ECG), and scheduling of MRI and liver biopsy. MRI and liver biopsy (if one has not been completed in the previous 6 months) should be performed with sufficient time to ensure results are received prior to the baseline visit.

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered the IP. A subject who had been designated a screen failure may be re-screened up to one time. However, re-screened subjects must begin the screening procedure again, must be re-consented,

and will be assigned a new subject number. Additionally, a subject's abnormal screening lab results may be repeated once for confirmation before designating a subject as a screen failure.

An investigative site may provide additional liver biopsy tissue to the central reader to be evaluated when the following criteria are met: 1.) the subject qualifies for the study on all inclusion/exclusion parameters except for liver histology, and 2.) the investigator's clinical assessment of the subject's local liver histology differs from that of the centrally read results, and 3.) the request to provide more tissue to the central reader for a second read must be requested before the patient is declared a screen failure. Any additional tissue samples provided must be read by the central reader and the central reader determines the final histological assessment.

7.1.2 Baseline (Visit 2)

Following the screening visit, subjects will return to the clinic within 56 days for Visit 2, (considered Week 0, Day 0), for baseline assessments including review of the inclusion/exclusion criteria, adverse events, and concomitant medications; physical examination with vital signs, weight, and waist/hip measurements; ECG; collection of blood and urine samples; stool assessment; and completion of the patient-reported outcome (PRO) EuroQol-5 Dimension-5 Level Questionnaire (EQ-5D-5L) survey. After completion of these assessments, subjects are randomized using Interactive Response Technology (IRT) to 1 of 4 treatment arms receiving volixibat 5 mg, 10 mg, or 20 mg, or PBO before the IA and then to 1 of the remaining arms after the IA. The initial supply of study drug is dispensed to ensure adequate daily dosing until the next scheduled study visit.

7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)

During the 48 weeks of double-blind treatment, 7 clinic visits are scheduled to occur as follows:

- Visit 3 - Week 2, Day 14 (+/- 3 days)
- Visit 4 - Week 4, Day 28 (+/- 3 days)
- Visit 5 - Week 8, Day 56 (+/- 5 days)
- Visit 6 - Week 12, Day 84 (+/- 5 days)
- Visit 7 - Week 24, Day 168 (+/- 7 days) – Interim Analysis
- Visit 8 - Week 36, Day 252 (+/- 7 days)
- Visit 9 - Week 48, Day 336 (+/- 7 days) – End of Study

Subjects are reminded not to eat and not to take their study drug on the day of scheduled study visits prior to completion of assessments. They should bring their study drug with them to the visit. All assessments should be completed at least 30 minutes prior to administration of the IP. IP should be taken 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat.

The Schedule of Assessments provided in Table 1 details the procedures to be completed at each visit. All Treatment visits (3 through 9) will include weight and waist/hip measurements, assessment of vital signs, and blood sampling for completion of biochemistry, hematology, serum glucose, lipid panel, HbA1c, and serum liver-related blood tests. At all visits, adverse events, and concomitant medications will be collected for all subjects, and female subjects of childbearing potential will have a urine pregnancy test. Subjects will return containers of unused study drug for assessment of accountability and compliance which will be documented in the IRT. New supplies of study drug will be dispensed at Visits 3 through 8.

Additional assessments will also occur less frequently during the Treatment Period for vitamin A, vitamin D, vitamin E, vitamin K via PT/INR, insulin, thyroid testing, urinalysis, ECG, physical examinations, stool assessment, and completion of the EQ-5D-5L.

An MRI will be repeated at Visit 7 for the 24-week IA and at Visit 9 (Week 48) for all subjects. A final liver biopsy will be performed at Visit 9 (Week 48) for all subjects unless a subject discontinues prior to Week 44, in which case the EOS liver biopsy is not required.

Sites that have supplied local slides for central reading for subject eligibility at Screening must also provide local liver histology slides for the subject to be centrally read at for the Week 48 EOS liver biopsy. The central reader will provide the final liver histology assessment for Week 48.

7.1.4 Follow-up (Visit 10)

The follow-up period for this protocol is 4 weeks after the last dose of study drug with a final Follow-up visit scheduled for Week 52. Procedures to be completed at this final visit include physical examination, weight, waist/hip measurements, vital signs, samples for biochemistry and hematology, serum glucose, lipid panel, HbA1c, serum liver-related blood tests, insulin, thyroid testing, stool assessment, and urine pregnancy test (for women of childbearing potential). Adverse events and concomitant medications will be recorded. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.5 Additional Care of Subjects after the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

All assessments and procedures are to be performed by the Investigator or a qualified designee who has been trained in the protocol. Assessments are to be performed according to the schedules shown in Table 1. If a subject terminates the study early, the CRC will make reasonable effort to perform the EOS and safety follow-up assessments and procedures for the subject's safety and well-being.

7.2.1 Demographic and Other Baseline Characteristics

Demographic details will be obtained at screening and recorded on the eCRF. Data collected will include age, gender, ethnicity, height, and weight.

7.2.2 Efficacy

7.2.2.1 Liver Biopsy

Liver biopsies will provide histologic data for confirmation of the diagnosis of NASH, assessment and grading of NASH activity, and scoring of steatosis, lobular inflammation, ballooning, as well as fibrosis and additional features (see Appendix 2 and Laboratory Manual for additional information).

7.2.2.2 MRI

The presence of ≥5% steatosis on screening MRI from a centrally read radiologist will be performed either during the screening period or within 6 months prior to the first visit. Steatosis is assessed by MRI hepatic fat fraction. The Week 24 MRI will be conducted for subjects in the IA set only.

7.2.3 Safety

7.2.3.1 Medical History and Medications

Medical history including important medical events and concomitant medication and illnesses will be obtained at screening and will be recorded on the eCRF. Any existing medical condition present prior to the time of randomization should be reported as medical history

7.2.3.2 Physical Examination

A complete physical examination will be performed with a thorough review of body systems at screening, baseline prior to randomization, and at study visits specified in Table 1. Physical examinations will include a review of the subject's general appearance, as well as evaluation of the body systems including:

- Eyes, ears, nose, throat
- Lymph nodes
- Cardiovascular
- Skin
- Abdomen
- Neurological
- Spine and extremities

Abnormalities identified at the screening visit (Visit 1) will be documented in the subject's source documents and on the medical history eCRF. Changes after the screening visit (Visit 1) will be captured as AEs on the AE eCRF page, if deemed clinically significant by the investigator.

Height will be measured at the screening visit only while weight and waist and hip circumference will be recorded at all study visits. BMI will be calculated programmatically.

7.2.3.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.3.4 Vital Signs

Vital signs include oral, temporal, or tympanic temperature, sitting blood pressure, pulse, and respiratory rate. Blood pressure should be determined by cuff (using the same method, the same arm, and in the same position throughout the study). Any deviations from baseline (Visit 2) vital signs which are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

7.2.3.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the central laboratory's normal procedures. Reference ranges supplied by the central laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Clinically significant findings should be evaluated for recording as adverse events on the eCRF. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Blood urea nitrogen (BUN)
- C4
- Lactate dehydrogenase (LDH)
- Magnesium (Mg)
- Phosphate (P)
- Potassium (K)
- Sodium (Na)
- Total and direct bilirubin

- Calcium (Ca)
- Bicarbonate (CO2)
- Chloride (Cl)
- Creatinine
- Creatine kinase
- Gamma glutamyl transferase (GGT)
- Glucose
- Total cholesterol
- Protein
- Triiodothyronine (T3)
- Thyroid-stimulating hormone (TSH)
- Triglycerides
- Uric acid

Hematology

- Hemoglobin
- Hematocrit
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Platelets
- Red blood cell (RBC)
- White blood cell (WBC) count with differential
- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Urinalysis

- Appearance (clarity and color)
- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopic examination of sediment
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen
- Oxalate

7.2.3.6 Additional Laboratory Assessments

Laboratory samples will be collected and assessed for:

- HIV testing and assessment of Hepatitis B/C including HBcAb, HBsAg, HBVDNA, and HCVAb (if HCVAb is positive an HCVRNA will be performed) will occur at the screening visit only

- Lipid panel including fasting total cholesterol, HDL-C, LDL-C, and triglycerides at each scheduled study visit (Visits 1 through 10)
- HbA1c will be tested at every scheduled visit (Visits 1 through 10)
- Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points at Visits 4, 7, and 9
- Insulin will be tested at screening, baseline, at Visit 4 and Visits 6 through 10
- HOMA-IR and HOMA-%B: will be calculated programmatically from serum glucose tested at screening, baseline, Visits 7, 9, and 10
- Vitamin A, Vitamin D, Vitamin E, and Vitamin K (via PT/INR) will be tested at baseline, and Visits 4, 7, and 9.
- Thyroid testing including TSH and T3 will be tested at screening, baseline, and Visits 4, 6, 7, 9, and 10
- C4 samples will be collected at baseline and Visits 6, 7, 9, and 10

7.2.3.7 Pregnancy Test

A urine pregnancy test is performed on all females of child-bearing potential at the screening visit (Visit 1), baseline visit (Visit 2), at each Treatment visit (Visits 3 through 9) and at the Final visit (Visit 10), or if pregnancy is suspected, or on withdrawal of the subject from the study. A positive urine pregnancy test must be followed with a serum pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Section 8.1.7.

7.2.3.8 Drug and Alcohol Testing

A urine screen for drugs of abuse and blood test for alcohol will be performed at screening and baseline as described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the eCRF database.

Any positive result for drugs of abuse at the screening or baseline visits may exclude the subject from further participation in the study. A positive test for drugs of abuse or alcohol done at the investigator's discretion discovered at any time during the study will be grounds for study discontinuation.

7.2.3.9 Electrocardiogram

An ECG (12-lead) will be performed at the times specified in Table 1 in accordance with the clinical site's standard practice(s) and equipment supplied by the CRC. Recordings of ECGs will be read locally at the clinical site by the investigator or designee. The ECG will include assessments of heart rate and PR, RR, QRS, and QT intervals. Identification of any clinically significant findings and/or abnormalities will be recorded on the eCRF.

7.2.3.10 Health-related Quality of Life Assessments

The EuroQol (EuroQol. 2016. Available at: http://www.euroqol.org. [Accessed 17 February 2016]) EQ-5D-5L ("EQ-5D") is a widely used standardized questionnaire that assesses generic HRQoL and is also recommended for health-economic evaluations. The EQ-5D includes two components: a descriptive profile and a visual analogue scale (VAS). The descriptive profile includes five dimensions (ie, pain/discomfort, mobility, usual activities, self-care and anxiety/depression), each with five levels (ie, no problems, slight problems, moderate problems, severe problems, extreme problems). An EQ-5D index can also be derived from the data which summarizes health status using a single value (ie, health-state utility). The psychometric properties of the EQ-5D-5L have been established and well documented (see Appendix 3).

7.2.3.11 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 146 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 146 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not the IP is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages

in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE eCRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of the IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of the IP, but the dyspepsia becomes severe and more frequent after first dose of the IP has been administered, a new AE of severe dyspepsia (with the appropriate date of onset) is recorded on the appropriate eCRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Appendix 4 provides criteria to assess the severity of liver-related adverse events.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to the IP for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the IP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the eCRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown.

8.1.4 Symptoms of the Disease Under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation of the disease and are part of the efficacy data to be collected in the study; however, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pre-treatment value observed closest to the start of study

treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.6 Criteria for Discontinuation of Treatment

Appendix 5 provides guidelines for discontinuation of treatment based on elevated ALT, AST, TB, and associated signs and symptoms.

8.1.7 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.4.

Any report of pregnancy for any female study participant (or the female partner of a male participant) must be reported within 24 hours to the Shire Global Drug Safety (formerly Pharmacovigilance and Risk Management) Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO medical monitor and the Shire clinical physician using the details specified in the Emergency Contact Information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-HCG test or ultrasound result will determine the pregnancy onset date.

8.1.8 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described

in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of the IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- Misuse – Intentional use of the IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol)

- Overdose – Intentional or unintentional intake of a dose of an IP exceeding a pre-specified total daily dose of the product.

- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the Volixibat Potassium (SHP626) Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Drug Safety (formerly Pharmacovigilance and Risk Management) Department and the Shire GCDL within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.8) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Drug Safety Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the Shire GCDL using the details specified in the Emergency Contact Information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence (whether considered to be related to the IP or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. 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.

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.4, and must be reported to the Shire Global Drug Safety Department and the Shire GCDL within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Drug Safety Department within 24 hours of the first awareness of the event.

8.2.4 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.5 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received the IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

8.2.6 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The CRO is responsible for notifying the relevant regulatory authorities as appropriate: USA central IRBs/EU central ECs of related, unexpected SAEs.

In addition the CRO is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP626-201 program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies

between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the eCRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Data that may potentially unblind the treatment assignment (eg, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513).

9.5 Planned Interim Analysis and Data Monitoring Committee

9.5.1 Planned Interim Analysis

Study enrollment will be paused after 92 subjects have been randomized in order for an IA to be conducted after at least 80 subjects have received 24 weeks of treatment. To protect study integrity, the IA will be performed by an independent statistician and statistical reporting group who are not involved in the clinical trial conduct and are not responsible for the final analysis of clinical trial data. The IA will be performed to evaluate the safety, tolerability, and efficacy of 3 doses of volixibat each compared to PBO and be used to drop 1 or 2 doses of volixibat, or to terminate the trial.

The criteria for deciding whether or not to continue randomizing to a particular volixibat dose following the Week 24 IA will be specified in the DMC charter. In general, randomization to any volixibat dose determined to be unsafe, intolerable or ineffective/futile at the IA will be discontinued. If all of the volixibat doses are determined to be intolerable or ineffective/futile the clinical trial will be terminated. If none of the volixibat doses are determined to be unsafe, intolerable or ineffective/futile then randomization to the volixibat dose that is the least promising or least helpful in characterizing the dose-response relationship (as specified in the DMC charter) will be discontinued. Tolerability will be assessed by comparing the number of discontinuations due to any one TEAE (presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain) between each volixibat dose group and PBO group. Efficacy will be assessed based on a reduction of steatosis (assessed by MRI-PDFF) and ALT by comparing the change from baseline in MRI-PDFF and percent change from baseline in ALT at Week 24 between each volixibat dose group and PBO group.

Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. That is, a subject receiving a dose to which randomization is stopped after the IA will continue to receive that same dose throughout the course of the study, and complete all required assessments, unless a dose is determined to be unsafe, in which case the affected subjects would be discontinued from the study and followed as per Section 4.5. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining volixibat dose groups or PBO group by IRT. Subjects will be randomized to 1 or 2 volixibat doses or to PBO. Thus, depending on the results from the IA, the study may be terminated, or one or two doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of IP.

9.5.2 Data Monitoring Committee

An independent DMC will be established to assess safety, tolerability, and efficacy during the study, as well as to ensure the validity and scientific merit of the trial. The DMC will monitor ongoing data generated by the study at regular intervals for the duration of the study. Their role is to protect the interests of the subjects in the study and of those still to be entered, by review of accumulating data generated in the study. If a safety concern is identified (eg, if either of the criteria are met in Section 4.5.4, Safety-related Stopping Rules), the DMC may recommend an action to the sponsor on further study conduct, including stopping the study at any time.

In addition, the DMC will review the results of the Week 24 IA and make recommendations concerning study discontinuation due to intolerability or futility, or the continued randomization of 1 or more volixibat dose groups and PBO.

The roles, responsibilities, and rules governing operation of the DMC will be discussed in full in a DMC charter. The DMC charter will define the primary responsibilities of the DMC; guide its activities, its relationship with other study components, its membership, and the purpose and timings of its meetings. It will provide the procedures for ensuring confidentiality, formal communication, and outline of the content of reports that will be provided by the DMC.

Appropriate summary statistics and data listings will be provided to the DMC by an independent statistician supported by an independent statistical reporting group not otherwise assigned to the study.

The recommendations made by the DMC to alter the conduct of the study or to stop the study will be forwarded to Shire for final decision. The implementation of any DMC recommendation is solely the responsibility of the sponsor. Shire will forward such decisions to regulatory authorities, as appropriate.

9.6 Sample Size Calculation and Power Considerations

These are the primary hypotheses that are being tested in this study:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.

- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

Response rates of 21% (PBO) and 45% (active) were reported in the FLINT trial (Neuschwander-Tetri et al. 2015) using the same primary end-point of reduction of NAS by at least two points (without worsening of fibrosis). Based on those response rates, 67 subjects per group completing the trial are needed for 80% power with a 10% type I error for a study comparing two volixibat doses to PBO. Holm multiplicity adjustment was included in the sample size estimate.

Approximately 677 subjects will be screened to enroll 266 subjects to achieve 201 completers. After at least 80 subjects (20/treatment arm – 3 active and 1 PBO) have received 24 weeks of treatment, an IA will eliminate at least one dose group based on the criteria provided to the DMC; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221

dropping out) subjects will be randomized if three arms (2 active and 1 PBO) continue; 208 (accounts for 20% dropping out) subjects are needed if 2 active doses are dropped at the IA.

9.7 Study Population

The Screened Set will consist of all subjects who have signed an informed consent.

The Randomized Set will consist of all subjects in the Screened Set who have been randomized into the study.

The Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an AE or reporting the absence of AEs).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

The Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the data cut time of the IA.

9.8 Efficacy Analyses

All efficacy analyses will be based on the FAS and all statistical tests will be 2-sided hypothesis tests performed at the 10% level of significance on those doses continued after the IA, accounting for the number of doses dropped at the IA.. Also, all confidence intervals will be 2-sided confidence intervals, unless otherwise stated.

9.8.1 Primary Efficacy Endpoint

The primary endpoint is the binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points, without worsening of fibrosis, from baseline NAS.

The FAS will be used to assess the primary efficacy endpoint. The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS will be analyzed. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of T2DM at baseline and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Subjects with missing data for the primary efficacy endpoint at the Week 48 visit will be considered as non-responders. Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level.

Analyses which explore the impact of the missing data on the primary efficacy endpoint will be conducted. These analyses may compare imputations of the missing values which favor the PBO and/or imputations which favor the active. In addition, analyses utilizing existing HFF and/or

ALT data may be performed. Further, if the pattern of missing values does not appear uniformly distributed among the treatment arms, imputation method(s) based on informative missingness may also be performed. Analyses investigating the impact of missing data will be detailed in the Statistical Analysis Plan.

9.8.2 Secondary Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the secondary efficacy endpoints. Binary secondary efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous secondary efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
- Change from baseline to Week 48 on hepatic steatosis as measured by MRI-PDFF.
- Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH CRN)
- Resolution of NASH (defined as total absence of ballooning [score = 0], absent or mild inflammation [score 0-1], steatosis can be present [score 0-3]) without worsening of fibrosis as assessed by liver histology at Week 48.
- Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
    - alanine aminotransferase (ALT)
    - aspartate aminotransferase (AST)
    - alkaline phosphatase (ALP)
    - gamma glutamyl transferase (GGT)
    - total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
    - fasting serum glucose levels
    - insulin levels
    - HbA1c

- Change from baseline to Week 48 on serum lipids measured by:
    - fasting total cholesterol
    - HDL-C
    - LDL-C
    - triglycerides

9.8.3 Exploratory Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the exploratory efficacy endpoints. Binary exploratory efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous exploratory efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F)
- Change from baseline to Week 48 on anthropomorphic measures:
    - body weight
    - BMI
    - waist circumference
    - waist-hip ratio
- Change from baseline to Week 48 in subjects with T2DM on homeostasis measured by:
    - Homeostasis model assessment -IR (HOMA-IR)
    - HOMA-beta cell function (HOMA-%B)

9.9 Safety Analyses

The Safety Analysis Set will be used to assess the safety endpoints.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. Treatment-emergent AEs are defined as AEs that start or worsen on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be calculated by SOC, by preferred term, and by treatment group.

Treatment-emergent AEs will be further summarized by severity and relationship to the IP. Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

9.10 Other Analyses

9.10.1 Health-related Quality of Life Analyses

- Change from baseline to Week 48 on the EQ-5D index (Appendix 3). The EQ-5D index will be derived using published UK-based preference weights. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from baseline to Week 48 on the EQ-5D VAS score. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from baseline to Week 48 in the proportion of subjects reporting having problems (none, slight, moderate, severe, extreme) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire. Proportions and 95% confidence intervals will also be generated for the baseline and Week 48 values as well as for change from baseline.

9.10.2 Stool Assessment

Stool hardness and number of evacuations will be assessed throughout the study. Stool hardness will be assessed for the softest evacuation within 24 hours of each clinic visit using the Bristol Stool Chart, a medical aid designed to classify the form of human feces into 7 categories where Type 1 is the hardest and Type 7 is the softest. Appendix 6 provides a sample of the Bristol Stool Chart. Number of evacuations within the past 24 hours prior to the clinic visit will be recorded at specified times as per Table 1. Descriptive statistics by treatment group will be presented for each time point at which the variable is measured.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements.

The name and address of each third party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of the IP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

10.1.3 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

10.1.5 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory

agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.do

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all the IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

eCRFs are supplied by the sponsor and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. eCRFs must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

All eCRF data must be endorsed by the investigator.

The CRA/study monitor will verify the contents of the eCRF against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, subject diary cards, original clinical laboratory reports, and histology and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, images, etc.). Non-study site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other USA national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in the IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB/EC and which received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor, prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor

or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an EC opinion can be the sponsor, the investigator, or for multicenter studies the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the sponsor or its designee has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor, the investigator or for multicenter studies the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All USA-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the sponsor or its designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market volixibat; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records

and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results / Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee, which is done for large, multicenter Phase 2-4 and certain other studies as determined by Shire. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 60 days prior to

submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

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12. APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	24 February 2016	Global
Amendment 1	8 April 2016	Global
Amendment 2	19 July 2016	Global
Amendment 3	22 March 2017	Global

APPENDIX 2 FATTY LIVER DISEASE HISTOLOGY SCORING

Diagnosis: NASH, Suspicious/Borderline NASH (type a or b), NAFLD, Not NAFLD

NAS SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Lobular Inflammation	0	no foci
		1	< 2 foci per 200x field
		2	2-4 foci per 200x field
		3	> 4 foci per 200 x field
	Ballooning	0	None
		1	Rare or diagnostically borderline
		2	Many or Prominent ballooned hepatocytes

SAF SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Ballooning	0	normal hepatocytes with cuboidal shape and pink cytoplasm
		1	clusters of hepatocytes with rounded shape and pale cytoplasm usually reticulated
		2	grade 1 plus enlarged hepatocytes, 2 x bigger than normal cells.
	Lobular Inflammation	0	none
		1	< 2 foci per 200x field
		2	>2 foci per 200x field

Steatosis 1,2,3 + Ballooning 1,2 + Lobular Inflammation 1,2 = NASH
Steatosis 1,2,3 +Ballooning 0 + Lobular Inflammation 0,1,2 = NAFLD
Steatosis 1,2,3 + Ballooning 1,2 + Lobular 0 = NAFLD
Steatosis 0 = no NAFLD

FIBROSIS SCORE	None	0
	Mild zone 3 perisinusoidal (requires trichrome)	1a
	Moderate Zone 3 perisinusoidal (visible on H&E)	1b
	Portal/periportal only	1c
	Portal, periportal and perisinusoidal	2
	Bridging	3
	Cirrhosis	4

References: Kleiner et al. 2005; Bedossa 2012

ADDITIONAL SCORING FEATURES

Expanded Balloon Score

0: None

1: Few Non classic

2: Few Classic

3. Many Classic

4: Severe Classic

Portal Inflammation

0: None

1: Minimal

2: Mild

3: More than Mild

Megamitochondria

0: None

1: Present

Acidophil Bodies

0: None or rare

1: Present

Fibrosis location if ≥ stage 2

Portal Predominant

Central Predominant

No predominance

Steatosis Zone

Zone 1 predominant

Zone 3 predominant

Azonal

Panacinar

Glycogenosis

None

Focal

Diffuse

APPENDIX 3 EQ-5D-5L HEALTH QUESTIONNAIRE UK SAMPLE ONLY, NOT FOR OFFICIAL USE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about	❑
I have slight problems in walking about	❑
I have moderate problems in walking about	❑
I have severe problems in walking about	❑
I am unable to walk about	❑

SELF-CARE

I have no problems washing or dressing myself	❑
I have slight problems washing or dressing myself	❑
I have moderate problems washing or dressing myself	❑
I have severe problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities	❑
I have slight problems doing my usual activities	❑
I have moderate problems doing my usual activities	❑
I have severe problems doing my usual activities	❑
I am unable to do my usual activities	❑

PAIN / DISCOMFORT

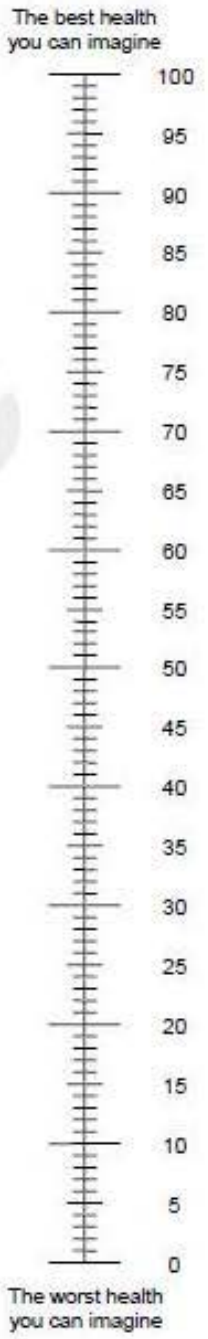
I have no pain or discomfort	❑
I have slight pain or discomfort	❑
I have moderate pain or discomfort	❑
I have severe pain or discomfort	❑
I have extreme pain or discomfort	❑

ANXIETY / DEPRESSION

I am not anxious or depressed	❑
I am slightly anxious or depressed	❑
I am moderately anxious or depressed	❑
I am severely anxious or depressed	❑
I am extremely anxious or depressed	❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 4 CRITERIA TO ASSESS SEVERITY OF LIVER-RELATED ADVERSE EVENTS

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
ALT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
AST	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Alkaline Phosphatase	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
GGT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Bilirubin	Normal	>1.0-1.5	>1.5-2.5	>2.5-5	>5

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
Note: Values expressed as multiples of the upper limit of the normal range (ULN).

APPENDIX 5 STOPPING RULES FOR LIVER-RELATED BLOOD TESTS

Table 1. Suggested Algorithm for Monitoring and Management of Drug-induced Liver Injury in Phase 2 and Phase 3 Nonalcoholic Steatohepatitis Studies in Patients With Normal or Elevated Baseline ALT[a]

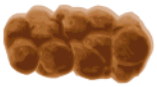
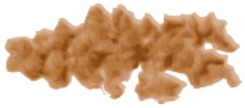
Treatment-Emergent ALT	Treatment-Emergent TBL	Liver Symptoms	Action
Normal baseline: ALT >5 × ULN Elevated baseline: ALT >3 × baseline or >300 U/L (whichever occurs first)	Normal For patients with Gilbert's syndrome: No change in baseline TBL	None	Repeat ALT, AST, ALP, TBL, in 2–5 days Follow-up for symptoms.
Normal baseline: ALT >8 × ULN Elevated baseline: ALT >8 × baseline or >500 U/L (whichever occurs first)	Normal Patients with Gilbert's syndrome: No change in baseline TBL	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >5 × ULN Elevated baseline: ALT >3 × baseline or >300 U/L (whichever occurs first)	TBL >2 × ULN For patients with Gilbert's syndrome: Doubling of direct bilirubin	None	Interrupt study drug. Initiate close monitoring and workup for competing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.
Normal baseline: ALT >5 x ULN Elevated baseline: ALT >3 x baseline or >300 U/L (whichever occurs first)	Normal or elevated	Severe fatigue, nausea, vomiting, right upper quadrant pain	Interrupt study drug. Initiate close monitoring and workup for completing etiologies. Study drug can be restarted only if another etiology is identified and liver enzymes return to baseline.

ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; TBL=total bilirubin; ULN=upper limit of normal.
[a]Baseline is the average of 2 screening ALT measurements.

Source: Chalasani and Regev 2016.

APPENDIX 6 BRISTOL STOOL CHART

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

PROTOCOL: SHP626-201

TITLE:	A Phase 2 Double-blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)
DRUG:	Volixibat potassium (SHP626)
IND:	123,847
EUDRACT NO.:	2016-000203-82
SPONSOR:	Shire Human Genetic Therapies, Inc. 300 Shire Way Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Philip Newsome MD Director of Centre for Liver Research University of Birmingham Edgbaston, Birmingham B15 2TT UK
NATIONAL/ COORDINATING INVESTIGATOR/UK	Guruprasad P Aithal MBBS, MD, FRCP, PhD Head of the Division, Nottingham Digestive Diseases Centre Director, NIHR Nottingham Digestive Diseases Biomedical Research Unit Nottingham University Hospitals and University of Nottingham Queen's Medical Centre Nottingham NG7 2UH
PROTOCOL HISTORY:	Amendment 2: 19 July 2016 Amendment 1: 8 April 2016 Original Protocol: 24 February 2016

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature:	**Date:** 19 July 2016
Melissa Palmer, MD, FAASLD Global Clinical Development Lead (GCDL) - Hepatology	

Investigator's Acknowledgement

I have read this protocol for Shire Study SHP626-201.

Title: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please hand print or type)	_____ _____ _____

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS VERSION

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **2**	**Amendment Date** **19 July 2016**	**Global/Country/Site Specific** **Global**
Section(s) Affected by Change	**Description of Change**	**Rationale**
General	Minor editorial changes	• Improved clarity, or correction
Study Synopsis	Inclusion and Exclusion criteria were revised. Endpoints and Statistical Analysis were revised.	• To improve clarity • To align with changes in the study protocol.
Table 1: Schedule of Assessments	Alcohol Test revised from Serum to Blood, Vitamins A and E were added, MRI time points were clarified, and window for Visit 10 was added.	• Improved clarity
Section 3.1 Study Design and Flow Chart	Specified that the interim analysis will be conducted after at least 80 subjects have completed 24 weeks of treatment.	• Improved clarity
Section 4.1 Inclusion Criteria	Inclusion criterion #4 regarding compliance with contraceptive requirements were revised.	• Improved clarity
Section 4.2 Exclusion Criteria	Acceptability of gastric sleeve was added to exclusion criterion #3.	• New text added, to provide clarity
Section 4.2 Exclusion Criteria	Exclusion #4 added text to include MRI in addition to liver biopsy for the weight change consideration.	• New text added, to provide clarity
Section 4.2 Exclusion Criteria	Typographical errors in criterion #7 regarding treatment with TZD or GLP-1 RA were corrected.	• Corrections to both terms
Section 4.2 Exclusion Criteria	Criterion #17 regarding use of anticoagulants was revised.	• Clarified that use of antiplatelet medications, including clopidogrel, will be allowed.
Section 4.2 Exclusion Criteria	Exclusion criterion #24 regarding pregnant women, women who plan to become pregnant, or men who plan to father a child during the study, was added.	• New text added, to provide clarity and supplement requirements for inclusion, stated in Section 4.1.
Section 4.4.1 Female Contraception	The list of acceptable methods of contraception was revised.	• Ensures alignment with methods considered to be "highly effective," ie, <1% failure rate per year.
Section 4.4.2 Male Contraception	Added that use of hormonal contraception by a female partner	• Ensures alignment with methods considered to be

	must be accompanied by a barrier method; also stipulated that male subjects must agree to use acceptable contraception for 30 days following the last dose of IP.	"highly effective," ie, <1% failure rate per year and addresses the issue of potential interaction of volixibat with hormones. • Further ensures safety for subjects and their partners.
Section 4.5.1 Subject Withdrawal Criteria	Revised criteria to clarify that subjects who become pregnant or demonstrate disease progression will be withdrawn;defined disease progression for this study.	• Improved clarity
Section 4.5.4 Safety-related Stopping Rules	Clarified sponsor consultation with DMC.	• Improved clarity
Section 5.2.1 Permitted treatment	Revised to clarify that use of antiplatelet medication is allowed.	• Improved clarity
Section 7.1.1 Screening (Visit 1)	Clarified that abnormal screening labs may be repeated before determining screen failure.	• Improved clarity
Sections 7.1.2 and 7.1.4	Added mention of stool assessment, to be consistent with Table 1.	• Improved clarity
Section 7.2.2.2 MRI	Clarified that the Week 24 MRI will be conducted for subjects in the IA set only, and deleted mention of Week 48, ie, all subjects will have EOS MRI.	• Change in analysis plan
Section 7.2.3.6 Additional Laboratory Assessments	Added Vitamins A & E, and clarified derivation of Vitamin K	• Additional assessments and clarity
Section 7.2.3.11 Volume of Blood to be Drawn from Each Subject	Revised blood volumes due to additional vitamin testing	• Additional assessments and clarity
Section 9.5.1 Planned Interim Analysis	This section heading was added and details regarding the planned interim analyses were added in this section. The specific rules for dose selection (and Appendix 6 information) were moved to Data Monitoring Committee (DMC) charter.	• This revised information improves clarity/ accuracy of the description of the interim analysis. • Appropriate reorganization of information.
Section 9.5.2 Data Monitoring Committee	This section heading was added and section was revised to improve clarity of processes.	• Improved clarity
Section 9.7 Study Population	This section was revised to improve clarity about the definition(s) of analysis sets.	• Improved clarity
Section 9.8.1 Primary Efficacy Endpoint	Removed the restriction that the impact of missing data will only be investigated if 10% or more of the data are missing. Added additional methods that may be used.	• Alignment with analysis plan
Section 9.8.2 Secondary Efficacy	This section was revised to provide	• Improved clarity

Endpoints	additional clarity about efficacy endpoints and associated analysis methods.	
Section 9.8.3 Exploratory Efficacy Endpoints	This section was revised to provide additional clarity about efficacy endpoints and associated analysis methods considered to be exploratory.	• Improved clarity
Section 9.9 Safety Analyses	This section was revised / reorganized to provide additional clarity.	• Improved clarity
Section 9.10.2 Stool Assessment	This section was revised to provide clarity about the analysis methods for these data.	• Improved clarity
Former Appendix 6: GENERAL PRINCIPLES AND SPECIFIC RULES FOR DOSE DISCONTINUATION AT 24 WEEK INTERIM ANALYSIS – GUIDANCE FOR DATA MONITORING COMMITTEE ONLY	This section was removed and all relevant information was placed in the DMC charter.	• Appropriate reorganization of information

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious Adverse Events (AEs) Required by the Protocol Form within 24 hours to:

Shire Global Pharmacovigilance and Risk Management Department

Preferred method: scan and e-mail to globalpharmacovigilance@shire.com
OR fax to +44(0) 1256 89 4715 (Global); or +1 866 557 4473 (North America)

AND

A copy of this form must also be sent to the Shire GCDL by fax or e-mail using the details below:

Melissa Palmer, MD, FAASLD, GCDL-Hepatology

fax number: +1 781 482 1822

e-mail: mpalmer@shire.com

For protocol- or safety-related issues during normal business hours 9:00 a.m. to 5:00 p.m. (Eastern Time), the investigator must contact the Contract Research Organization (CRO) Medical Monitor:

ICON Medical Monitor: Anthony Japour, MD

Telephone number: +1 215 616 6439
Fax number: +1 215 616 3096
Mobile number: +1 267 429 6601
E-mail address: Anthony.Japour@iconplc.com

For protocol- or safety-related issues outside of normal business hours, the investigator must contact ICON's 24/7 Medical Emergency Coverage:

Chargeable global telephone number: +1 919 674 5468

NOTE: Investigative sites will be provided country-specific toll-free telephone numbers. Please refer to this document, as applicable. Countries without a toll-free number will need to dial the chargeable number noted above.

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

Please use the information below as applicable to report the Product Quality Complaint:

Origin of Product Quality Complaint	**E-mail Address**
North and South America	PQC@shire.com
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Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
1-888-300-6414 or 1-800-828-2088

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ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPPT	activated partial thromboplastin time
ASBT	apical sodium-dependent bile acid transporter
ASBTi	ASBT inhibitor
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BA	bile acid
BMI	body mass index
C4	7-alpha-hydroxy-4-cholesten-3-one
CRA	clinical research associate
CRC	clinical research center
CRO	contract research organization
DMC	data monitoring committee
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimension-5 Level Questionnaire
ECG	electrocardiogram
EOS	end of study
EU	European Union
FAS	full analysis set
FBA	fecal bile acid
FDA	Food and Drug Administration
FXR	farnesoid X receptor
GCDL	Global Clinical Development Lead
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP-1 RA	glucagon-like peptide-1 receptor agonists
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBVDNA	hepatitis B virus deoxyribonucleic acid
HCV	hepatitis C virus

HCVAb	hepatitis C virus antibody
HCVRNA	hepatitis C virus ribonucleic acid
HDL-C	high-density lipoprotein-cholesterol
HFD	high fat diet
HFF	hepatic fat fraction
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HOMA-%B	homeostasis model assessment in β-cell function
HOMA-IR	homeostasis model assessment insulin resistance
HRQoL	health-related quality of life
IA	interim analysis
IAS	interim analysis set
ICH	International Conference on Harmonisation
IND	investigational new drug
INR	international normalized ratio
IP	investigational product
IR	insulin resistance
IRB	Institutional Review Board
IRT	interactive response technology
LDL-C	low-density lipoprotein-cholesterol
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MOA	mechanism of action
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NOAEL	no observed adverse effect level
PBC	primary biliary cirrhosis
PBO	placebo
PDFF	proton density fat-fraction
PO	by mouth

POC	proof of concept
PRO	patient-reported outcome
PSC	primary sclerosing cholangitis
PT	prothrombin time
PVRM	Pharmacovigilance and Risk Management
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAF	steatosis, activity, and fibrosis score
SAP	statistical analysis plan
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TA	therapeutic area
TB	total bilirubin
TEAE	treatment-emergent adverse event
TGR5	transmembrane G protein-coupled receptor
TPN	total parenteral nutrition
TSH	thyroid-stimulating hormone
TZD	thiazolidinediones
ULN	upper limit of normal
USA	United States of America
VAS	visual analogue scale

STUDY SYNOPSIS

Protocol number: SHP626-201	**Drug:** Volixibat potassium (SHP626)
Title of the study: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)	
Number of subjects (total and for each treatment arm): Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 placebo [PBO]) a 24-week interim analysis (IA) will eliminate at least one dose group based on the criteria provided to the Data Monitoring Committee; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue. Fewer subjects are needed if more than one SHP626 dose is dropped.	
Investigator(s): Multicenter study	
Site(s) and Region(s): Anticipated regions -US, Canada, and EU Estimated number of sites planned – 60 to 80	
Study period (planned): July 2016 to July 2019	**Clinical phase:** 2

Objectives:

Primary: To evaluate the effect of volixibat compared to PBO on liver histology

Secondary:

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual nonalcoholic fatty liver disease (NAFLD) activity score (NAS) components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

Rationale: Currently, there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its mechanism of action (MOA) and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

Investigational product, dose, and mode of administration:

- Volixibat 5, 10, and 20 mg and matched PBO capsules by mouth (PO) once daily (QD). Investigational product (IP) should be given 30 minutes prior to the first meal of the day containing approximately 10-20

grams of fat. Also see Section 6.2.3 (Dosing).

- Identical PBO will be used as comparator
- Subjects should take the IP at the same time each day and should not take more than one dose in a day if they miss a dose

Methodology:

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have received 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

Inclusion and exclusion criteria:

Inclusion Criteria:

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally-authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and 30 days following the last dose of the IP.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F1-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

Exclusion Criteria:

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV (defined as HCVAb positive without a current or prior history of a detectable HCVRNA) will be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the IP (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver

biopsy and/or MRI within 6 months of screening, but experienced a weight change ≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.

5. Contraindications to MRI (ie, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation.
8. Uncontrolled diabetes defined as HbA1c of ≥9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to qualifying liver biopsy, or initiated any time after qualifying liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST >5 times upper limit of normal (ULN) at screening.
12. Serum ALT >5 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3.
15. Total bilirubin (TB) ≥2.0 x ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$L
17. Medical history of impaired hemostasis or current use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie, 81mg, aspirin (ASA) or clopidogrel (Plavix), will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.

25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject has failed screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
31. Subjects who are employees at the investigational site.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 56 days
- Planned duration of enrollment period: 364 days
- Planned duration of treatment period: 336 days
- Planned duration of follow-up: 28 days

Endpoints and statistical analysis:

Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an adverse event (AE) or reporting the absence of AEs).

Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the time of the data cut for the IA.

Screened Set will consist of all subjects who have signed an informed consent.

Efficacy Endpoints:

- ***Primary:*** Binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS.
- ***Secondary:***
    - Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
    - Change from baseline to Week 48 on hepatic steatosis as measured by MRI-HFF.
    - Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH Clinical Research Network (CRN))
    - Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at week 48.
    - Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
        - alanine aminotransferase (ALT)
        - aspartate aminotransferase (AST)
        - alkaline phosphatase (ALP)
        - gamma glutamyl transferase (GGT)

- total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
  - fasting serum glucose levels
  - insulin levels
  - HbA1c
- Change from baseline to Week 48 on serum lipids measured by:
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

Primary hypotheses:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.
- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

The FAS will be used to assess the primary efficacy endpoint. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of Type 2 diabetes mellitus (T2DM) at baseline, and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level. Histology will be read by one central hepatohistopathologist who will use the NASH CRN standard scoring system – NAS. The SAF Steatosis (S), Activity (A), and Fibrosis (F) scoring system will be determined for exploratory purposes.

The Safety Analysis Set will be used to assess the safety endpoints including AEs (including changes from baseline in physical examination findings), vital signs, ECGs, and clinical laboratory tests (chemistry, hematology, coagulation and urinalysis).

AEs will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as AEs that started or worsened on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be presented by system organ class, preferred term, and by treatment group. TEAEs will be further summarized by severity and the relationship to the IP. AEs related to the IP, AEs leading to withdrawal, SAEs, and death will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

For safety parameters, baseline is defined as the last assessment prior to the first dose of the IP.

Planned Interim Analysis:

An IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO.

Tolerability will be assessed by the number of discontinuations due to any one TEAE, presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Efficacy at the IA will be based on reduction of steatosis or ALT. Steatosis is assessed by MRI-HFF. Depending on the results from the IA, one or more doses of volixibat may be discontinued or the study may be terminated. If the study is not terminated, subjects will receive a total of 48 weeks of the IP.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Informed consent	X									
Inclusion/exclusion criteria	X	X (review)								
Demography, medical & medication history	X									
Physical examination	X	X					X		X	X
Height[c], weight[d], waist circumference and waist:hip ratio	X	X	X	X	X	X	X	X	X	X
Vital signs[e]	X	X	X	X	X	X	X	X	X	X
PRO (EQ-5D-5L)		X					X		X	
Liver biopsy[f]	X								X	
MRI[g]	X						X[h]		X	
ECG (12-lead)	X	X				X			X	
Biochemistry and Hematology[i]	X	X	X	X	X	X	X	X	X	X
Serum Glucose [i,j]	X	X	X	X	X	X	X	X	X	X
Urinalysis[k]	X	X							X	
Urine Drug and Blood Alcohol Tests	X	X								
Urine Pregnancy Test[l]	X	X	X	X	X	X	X	X	X	X
Lipid Panel[i,m]	X	X	X	X	X	X	X	X	X	X
Coagulation Panel[i,n]	X	X		X			X		X	
Vitamins A, D, and E [i]	X	X		X			X		X	

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
HbA1c[i]	X	X	X	X	X	X	X	X	X	X
Serum Liver-Related Blood Tests[i,o]	X	X	X	X	X	X	X	X	X	X
Insulin[i]	X	X		X		X	X	X	X	X
HIV, Hepatitis B/C[i,p]	X									
Thyroid testing[i,q]	X	X		X		X	X		X	X
C4 Sampling		X				X	X		X	X
IRT Accessed	X	X	X	X	X	X	X	X	X	X
Randomization		X								
IP Dispensed[r]		X	X	X	X	X	X	X		
IP Returned/Accountability & Compliance Assessed			X	X	X	X	X	X	X	
Stool Assessment[s]		X	X			X		X	X	X
Adverse Events[t]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X

ECG=electrocardiogram; EOS=end of study; HOMA=Homeostasis Model Assessment; IA=interim analysis; IP=investigational product; IRT=Interactive Response Technology; MRI=magnetic resonance imaging; PRO=patient-reported outcome.

[a] Visit Windows (calculated from Visit 2): Bi-weekly (Visits 3-4): +/- 3 days; Monthly (Visits 5-6, Visit 10): +/-5 days; Tri-monthly (Visits 7-9/DC): +/- 7 days.

[b] Subjects will be reminded not to eat prior to their scheduled visit. Additionally, during the double-blind treatment period, they should not take their study drug prior to the visit. They should bring their study drug with them to the visit to take 30 minutes prior to their first meal of the day containing approximately 10-20 grams of fat. Also see Section 6.2.3

[c] Height to be measured at screening only.

[d] BMI to be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7 and 9/DC.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9 EOS	10 In-Clinic
Week	-8 to 0	0	2	4	8	12	24	36	48	52
Study Day	-56 to -1	0	14	28	56	84	168	252	336	364

[e] Vital signs to include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate.

[f] Biopsy performed within 6 months of screening can be used. All biopsies will be centrally read by a hepatic histopathologist.

[g] MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.

[h] - MRI at Visit 7 (Week 24) for the Interim Analysis Set only.

[i] All blood tests are fasting blood tests.

[j] HOMA-IR and HOMA-%B: will be calculated programmatically by the sponsor or designee for the following visits: screening (Visit 1), baseline (Visit 2), Visits 7, 9/DC, and 10.

[k] Urinalysis to include oxalate testing.

[l] For all females of child-bearing potential (FOCP). Positive on-site urine dipstick results must have serum β-HCG testing performed by central lab. Additional testing can be performed at the investigator's discretion.

[m] Lipid Panel includes fasting total cholesterol, HDL-C, LDL-C, and triglycerides.

[n] Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points to assess vitamin K level.

[o] Serum Liver-Related Blood Tests include ALT, AST, ALP, GGT, and total bilirubin.

[p] Hepatitis B/C testing includes HBcAb, HBsAg, HBVDNA and HCVAb, HCVRNA, respectively.

[q] Thyroid testing includes thyroid stimulating hormone (TSH) and triiodothyronine (T3).

[r] Investigational product may be dispensed at an unscheduled visit outside of this schedule as needed to replace lost or damaged product.

[s] Subjects will be queried about the number of stool evacuations during the 24- hour period before the clinical research center (CRC) visit and asked to describe the consistency of the softest stool during that 24- hour period using the Bristol Stool Chart.

[t] Adverse events will be collected beginning from the signing of informed consent. All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

1. BACKGROUND INFORMATION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide (Vernon et al. 2011), and is estimated to occur in 30-40% of adults in the United States and up to 30% of European adults. These numbers approach 95% in those with morbid obesity (Mathus-Vliegen et al. 2012). NAFLD ranges from simple steatosis, which is typically nonprogressive, to nonalcoholic steatohepatitis (NASH), which has a 20% likelihood of progression to advanced disease including fibrosis, cirrhosis and its complications including liver failure and the need for a liver transplant (Angulo 2002). Hepatocellular carcinoma is also a complication of NASH that may occur with or without the presence of cirrhosis (Torres et al. 2012). NAFLD is typically associated with type 2 diabetes mellitus (T2DM) (Adibi et al. 2007; Loomba et al. 2012), central or visceral obesity (Souza et al. 2012), dyslipidemia (Assy et al. 2000; N.C.E.P. 2002), and hypertension (Donati et al. 2004). Together these conditions comprise the metabolic syndrome, and NASH is considered to be the hepatic component of this syndrome (Hamaguchi et al. 2005; Neuschwander-Tetri 2005; Marchesini et al. 2005). Notably, NAFLD is a clinical condition occurring in individuals who do not drink excessive alcohol (>20 grams/day), yet have hepatic histology which is indistinguishable from that seen with alcoholic excess. The pathophysiology of NASH is likely multifactorial and may include combinations of metabolic, genetic, environmental, and gut microbial factors.

Most individuals with NASH are asymptomatic or have nonspecific symptoms such as fatigue. They typically first come to medical attention incidentally following routine blood testing or on imaging studies performed routinely or during the evaluation of an unrelated condition. While ultrasound and magnetic resonance imaging (MRI) can detect the presence of steatosis (Reeder et al. 2011), a liver biopsy is required to diagnose NASH and the extent of liver fibrosis.

1.1 Indication and Current Treatment Options

There are currently no drugs approved for the treatment of NASH and it is estimated that there are between 6-16 million people in the United States with NASH, of which 600,000 have severe disease (Williams et al. 2011 and Torres and Harrison 2008), with similar percentages reported throughout most areas of the world (World Gastroenterology Organisation 2012). Treatment of associated metabolic comorbidities, weight reduction, and incorporation of an exercise routine remain the cornerstone of management. However, lifestyle changes are seldom successful. Thus, NASH represents a disease with an unmet medical need that is growing at an epidemic rate, and that if untreated, carries a risk of significant morbidity and mortality.

1.2 Product Background and Clinical Information

Volixibat potassium (SHP626; formerly LUM002), hereafter referred to as volixibat, is a highly selective inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that is being evaluated for the treatment of NASH.

Bile acids (BA) promote bile flow, activation of digestive enzymes, and micellization of fats and fat-soluble vitamins, thereby permitting their intestinal absorption. BAs serve as signaling

molecules acting via receptors, such as farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR5), in the intestine, liver and other tissues which play an important role in regulating insulin homeostasis. (Halilbasic et al. 2013). Lipid peroxidation and oxidant stress have been proposed as one link between the accumulation of fat and subsequent injury (Day and James 1998). All of these metabolic actions have important effects to prevent the ongoing liver damage in NASH.

High fat diet (HFD) fed mice treated with an ASBT inhibitor (ASBTi) (SC-435 a surrogate SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved insulin resistance (IR), and decreased NAS, predominantly steatosis. In addition the BA pool reflected increases in BA that were agonists to FXR and decreases in levels that were antagonist to the FXR (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015). Miethke and colleagues (2016) found that pharmacological inhibition of the ileal, ASBT (SC-435 the surrogate molecule of volixibat), blocked progression of sclerosing cholangitis in mdr2–/– mice. Beneficial effects in liver histology in these mice included a reduction in the severity of hepatic fibrosis, a decrease which correlated with a reduction of hepatic profibrogenic gene expression. While very encouraging, whether or not the findings in this mouse sclerosing cholangitis model can translate to histologic improvements in patients with NASH is unknown.

Volixibat, as a potent inhibitor of ASBT, increases BA excretion and facilitates signaling in the intestine that regulates serum and hepatic BA concentrations, glucose metabolism, serum cholesterol and fatty acid metabolism in the liver. The combined events resulting from inhibiting BA reuptake are hypothesized to have a positive metabolic, anti-inflammatory, anti-steatotic, and potentially antifibrotic effect that will lead to a therapeutic benefit for patients with NASH.

One of the factors contributing to the pathogenesis of NASH is abnormal cholesterol metabolism and the accumulation of free cholesterol in the liver. The free cholesterol is directly toxic to hepatocytes, which leads to inflammation and fibrosis (Musso et al. 2013). One treatment approach is to remove the cholesterol from the liver to decrease and possibly reverse the damage to the hepatocytes. This is one of the mechanisms by which SHP626 will treat NASH.

Due to the mechanism of action (MOA), volixibat is under development for the treatment of NASH and may also be able to improve the metabolic syndrome that is associated with NASH. Volixibat inhibits ASBT therefore BAs are excreted in the feces and this loss forces the liver to synthesize new BA which utilizes cholesterol in the liver and serum. Volixibat also has the potential to reduce IR, which is considered to be the most common underlying risk factor for the development of NASH (Pagano et al. 2002; Sanyal et al. 2001).

Volixibat was initially being evaluated for its use as an intervention for dyslipidemia. As a result of initial observations in animals that serum low-density lipoprotein cholesterol (LDL-C) and total liver cholesterol content could be decreased after administration of volixibat, additional work was undertaken to support the safety and tolerability of the drug in healthy volunteers. Clinical and non-clinical studies have demonstrated very low systemic exposure across species. Oral administration of volixibat at doses up to 300 mg once daily and 50 mg administered daily for 14 days to healthy male subjects was generally safe. A Phase 1 study revealed that volixibat is tolerated at the 10 mg dose for 28 days and that there were trends towards increasing high-

density lipoprotein cholesterol (HDL-C), decreasing LDL-C, and decreasing fasting glucose in patients with T2DM (LUM002-101 trial).

A healthy volunteer study investigating 12 days of varying levels of repeat doses of volixibat has provided key pharmacodynamic information for dose selection as measured by the effect of volixibat on excreted fecal bile acid (FBA) levels (SHP626-101). In addition, the study investigated safety and tolerability over the dose range that was considered for this Phase 2 study.

Data from Phase 1 studies support the position that volixibat is basically a non-absorbed drug that works locally in the GI tract and results in virtually no systemic exposure. Thus pharmacokinetic sampling will not be done in this study.

The current proof of concept (POC) Phase 2 trial will evaluate the safety, tolerability, and efficacy of three doses of volixibat (5, 10, and 20 mg) in adult subjects with NASH. Due to the MOA of volixibat, efficacy will be assessed at an interim analysis (IA) by a change of steatosis from baseline to Week 24 compared to placebo (PBO). While the gold standard for quantification of steatosis has historically been an invasive liver biopsy, quantitative magnetic resonance (MR) imaging–based biomarkers for liver fat have evolved rapidly over the last decade, and are increasingly being incorporated into NASH clinical trials. Both MR spectroscopy (S) and MR imaging (I) proton density fat-fraction (PDFF) provide non-invasive means of quantifying intrahepatic lipid content (Reeder et al. 2012). Both techniques have been shown to be accurate, reproducible with a low degree of variability in interpretation, cost-effective and reliable biomarkers of quantitative hepatic fat (Roldan-Valadez et al. 2010; Urdzik et al. 2012; Raptis et al. 2012). Importantly, studies demonstrate a close correlation with steatosis grade histologically (Qayyum et al. 2005; Schwenzer et al. 2009).

In a study of 51 adult subjects with NAFLD, PDFF correlated well with the grade of histologic steatosis, as the mean fat-fraction values of 8.9%, 16.3%, and 25.0% corresponded to histologic steatosis grades 1, 2, and 3, respectively ($P < .0001$) (Permutt et al. 2012). Thus, MRI will be utilized to evaluate the degree of steatosis change from baseline in this study during the IA. The Mozart trial was a randomized, double-blind, PBO-controlled trial of 50 patients with NASH who were randomized to 24 weeks of either ezetimibe or PBO, evaluating the reduction of liver fat by MRI-PDFF as well as by histology. Results revealed that compared to histologic non-responders, histologic responders, defined as a two-point reduction in NAFLD activity score (NAS) without worsening fibrosis, had a statistically significant reduction in net MRI-PDFF of $-4.1\% \pm 4.9$ vs. $+0.6\% \pm 4.1$ ($P <0.036$) with a mean percent change of $-29.3\% \pm 33.0$ vs. $+2.0\% \pm 24.0$ ($P < 0.004$), respectively (Loomba et al. 2015). Thus, in the current trial during the IA, a $\geq$5% steatosis reduction for an active dose compared to PBO will be a clinically meaningful change after 24 weeks of therapy.

Intrahepatic lipid content of less than 1% is considered to be within the normal range (Springer et al. 2015), however, from a study of 2349 people in a general population undergoing MRS, it was concluded that a PDFF value of 5.56% represented the upper limit of the normal range, as determined from the 95th percentile of PDFF in 345 individuals who were not at increased risk for hepatic steatosis (Szczepaniak et al. 2005). Thus, in the current trial, similar to

other NASH trials utilizing MR for evaluation, an MRI ≥5% steatosis will be used as an inclusion criterion (Loomba et al. 2015).

1.3 Benefits and Risks

By virtue of volixibat's ability to inhibit ASBT bile acid reabsorption, there is an increase in BA excretion and signaling in the intestine that results in improvements in glucose metabolism and changes in cholesterol and fatty acid synthesis in the liver. Recently, HFD fed mice treated with an ASBTi (SC-435 a surrogate of SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved IR, and decreased NAS (predominantly steatosis). In addition, these HFD-fed mice did not gain weight when treated with SC-435, in spite of consuming increased calories. Finally, the BA pool in these mice changed to predominantly FXR agonist (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015).

These metabolic actions and preclinical results may prove to be clinically relevant to subjects with NASH.

NASH has recently received considerable attention as awareness of the problem of liver damage and prevalence of the disorder has increased, paralleling the obesity epidemic. Consequences of liver damage are detrimental and can lead to liver failure, hepatocellular carcinoma, and the need for liver transplantation. There is no currently approved medical therapy for NASH. The large unmet medical need and the increased medical resource burden have led to the search for potential therapies to treat NASH.

Nonclinical testing established that the no observed adverse effect level (NOAEL) for volixibat in rats and dogs following 13 weeks of once-daily administration were 1000 and 500 mg/kg/day, respectively. Similarly, testing confirmed that the NOAEL for volixibat in a 6-month study in rats and a 9-month study in dogs were 1000 and 500 mg/kg/day, respectively. In both cases, these were the highest doses tested. Genotoxicity testing has yielded negative findings.

Volixibat is minimally absorbed. The pharmacokinetic profiles performed in clinical studies completed to date repeatedly suggest negligible systemic exposure. Furthermore, there has been no observation of clinically relevant changes in fat absorption parameters such as those related to fat-soluble vitamins.

The most frequent TEAEs in the Phase 1 studies were GI and were considered mechanism-based due to elevated BA concentrations in the colon. The percentage of subjects reporting at least 1 TEAE in the GI disorders SOC generally increased with an increasing volixibat dose level (Part 1 Study TDU10632 and Study LUM002-101). Most TEAEs were mild in intensity, and none were assessed as severe.

In the multiple dose studies, the most commonly reported TEAEs in subjects (both healthy and with T2DM) who received volixibat for the longest duration of 28 days in Study LUM002-101 included diarrhea and abdominal pain. The most commonly reported TEAEs in subjects receiving 50 mg volixibat for 14 days (part 3 Study TDR10633) were diarrhea and GI pain.

Overall, there were 2 SAEs (ALT increased and retinal detachment), both of which led to the discontinuation of volixibat. In part 3 of the initial Phase 1 study (TDU10633), 1 subject dosed with 50 mg volixibat for 13 days was withdrawn from the study due to a mild TEAE (which became a SAE due to prolonged hospitalization) of ALT increased that was considered related to volixibat . The subject's ALT level returned to normal after discontinuation of volixibat. A second subject dosed with 10 mg volixibat for 12 days in Study LUM002-101 reported a moderate SAE of ablation of the retina with a bleed in the vitreous body of the right eye that was considered not related to volixibat.

Overall, 3 subjects, all dosed with 5 mg volixibat in Study LUM002-101, discontinued volixibat due to non-serious TEAEs: 1 due to a related TEAE of mild hemorrhagic diarrhea, 1 due to an unrelated TEAE of moderate Epstein-Barr virus infection, and 1 due to mild related TEAEs of diarrhea and anal erosion.

Overall, the observed AEs attributable to volixibat have been self-limited as would be expected given the local MOA of ASBT inhibition in the terminal ileum. Generally, among subjects who experienced GI TEAEs, the events have been mild and diminished over the course of treatment. Please refer to the investigator's brochure for additional information.

Volixibat is a novel drug candidate, demonstrating limited systemic exposure across species with the potential to affect important metabolic pathways associated with NASH. The overall safety, tolerability, and preliminary activity of volixibat in available clinical trials suggest that further investigation is warranted and that there is a positive benefit to risk profile.

Always refer to the latest version of the Volixibat Potassium (SHP626) Investigator's Brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of volixibat.

See Appendix 1 for protocol history, including all amendments.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Currently there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its MOA and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

2.2 Study Objectives

2.2.1 Primary Objective

To evaluate the effect of volixibat compared to PBO on liver histology

2.2.2 Secondary Objectives

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual NAS components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

2.2.3 Exploratory Objectives

- To explore the effect of volixibat compared to PBO on liver histology (measured by individual SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F))
- To explore the effect of volixibat compared to PBO on anthropometric measures (body weight, body mass index (BMI), waist circumference and waist-hip ratio)
- To explore the effect of volixibat compared to PBO on homeostasis model assessment-IR (HOMA-IR) and HOMA-beta cell function (HOMA-%B) in subjects with T2DM
- To explore the effect of volixibat compared to PBO on BA synthesis (7-alpha-hydroxy-4-cholesten-3-one [C4])

- To explore the effect of volixibat on patient-reported health-related quality of life (HRQoL) and overall health status.

3. STUDY DESIGN

3.1 Study Design and Flow Chart

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after at least 80 subjects have 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio such that a target of 266 subjects is achieved (221x1.2 is approximately 266). The 221 subjects include 201 subjects for the three arms analyzed at 48 weeks plus an additional 20 subjects to account for one dose dropped at the IA). Attempt will be made to perform the IA before any subject has had their 48-week post-treatment liver biopsy, although this will be dependent upon the rate of enrollment and dropout.

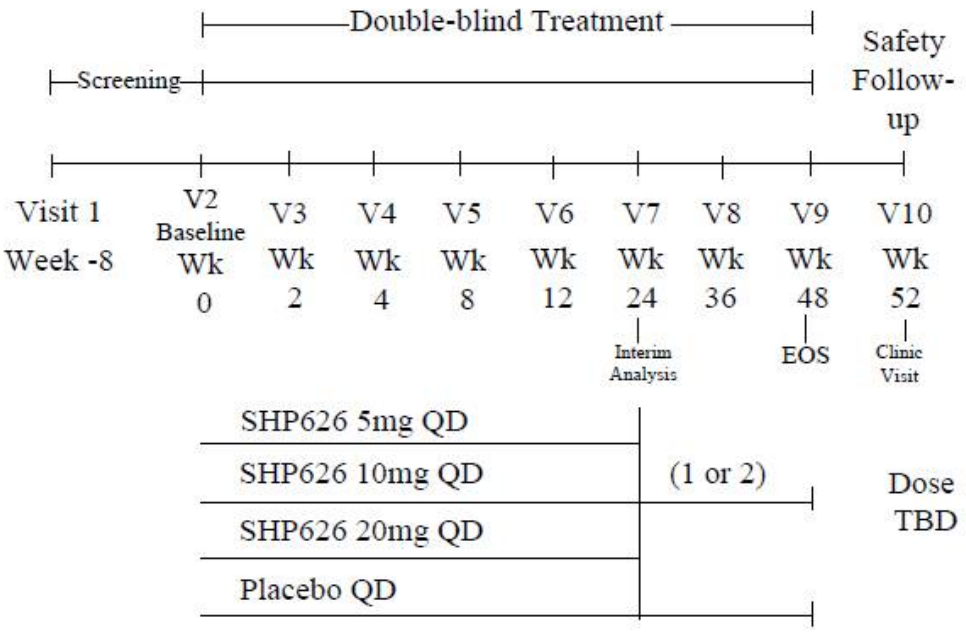
There will be up to 3 periods (screening, treatment, and follow-up) and an IA. The duration of the Treatment period will be 48 weeks, with an IA at Week 24. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

The IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. Study enrollment will be paused after 92 subjects have been randomized. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO.

Once decisions as to dose elimination are made, study enrollment will be reopened and additional sites may be added. Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining dose groups (noted by "[1 or 2]… Dose TBD" in Figure 1) or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of investigational product (IP).

The study will be conducted over 3 periods: screening (8 weeks), treatment (48 weeks), and follow-up (4 weeks), with an IA at 24 weeks as outlined in Figure 1.

Figure 1: Study Design Flow Chart



3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 364 days. The last visit is an in-clinic safety follow-up visit. The study will be completed in approximately 3 years.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at approximately 60 to 80 clinical sites in the USA, Canada, and EU.

4. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first screening visit.
4. Male, or non-pregnant, non-lactating female, who is sexually active and who agrees to comply with the contraceptive requirements of the protocol, or females of non-childbearing potential. Males and females of child-bearing potential who are sexually active must agree to use acceptable contraception during the study and for 30 days following the last dose of the IP as described in Section 4.4.
5. Presence of ≥5% steatosis on screening MRI from a centrally read radiologist performed either during the screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F1-F3) from a centrally read liver biopsy performed either during the screening period or within 6 months prior to the first visit with a NAS of ≥4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met.

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen [HBsAg] positive and/or HBVDNA positive; subjects who are hepatitis B core antibody [HBcAb] positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV [defined as HCVAb positive] without a current or prior history of a detectable HCVRNA) may be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.

3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the investigational product (IP) (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band or gastric sleeve is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥5% after qualifying liver biopsy and/or MRI performed. If the subject had a liver biopsy and/or MRI within 6 months of screening, but experienced a weight change of ≥5% since the date of liver biopsy and/or MRI, the liver biopsy and/or MRI must be repeated at screening.
5. Contraindications to MRI (eg, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptor agonists (GLP-1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation (refer to Section 5.2.1, Permitted Treatment).
8. Uncontrolled diabetes defined as HbA1c of ≥9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to screening. Also refer to Section 5.2.2, Prohibited Treatment.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to liver biopsy, or initiated any time after liver biopsy performed, including the use of total parenteral nutrition (TPN). Also refer to Section 5.2.2, Prohibited Treatment.
11. Serum AST > 5 times upper limit of normal (ULN) at screening.
12. Serum ALT > 5 times ULN at screening.
13. Elevated serum creatinine ≥2.0 mg/dL.
14. International normalized ratio (INR) >1.3
15. TB ≥2.0 x ULN at screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count $<130 \times 10^9$/L
17. Medical history of impaired hemostasis or use of anticoagulant medication (use of antiplatelet medications, such as low-dose, ie 81 mg, aspirin (ASA) or clopidogrel (Plavix) will be allowed).
18. Uncontrolled thyroid disease.

19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Refer to Section 7.2.3.8, Drug and Alcohol screening. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. Females who are pregnant, planning to become pregnant, or are breastfeeding, or males who are planning to father a child during study participation.
25. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
26. Known positivity for human immunodeficiency virus (HIV) infection.
27. Cancer within 5 years of screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
28. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
29. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
30. Subject has failed screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
31. Subjects who are employees at the investigational site.

4.3 Restrictions

Subjects must adhere to the following restrictions for the duration of the study:

- Subjects must remain compliant with inclusion/exclusion criteria.
- Subjects should not become pregnant, father a child, or nurse/breastfeed a baby.
- Subjects should be encouraged to adhere to the same exercise routine and a healthy diet throughout the study.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of child-bearing potential should use an acceptable form of contraception. Females of child-bearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of the IP. If hormonal contraceptives are used they should be administered according to the package insert.

Female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and age ≥51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of child-bearing potential with a negative urine and/or serum β-human chorionic gonadotropin (β-HCG) pregnancy test each study visit. Females of child-bearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of highly-effective contraception.

Acceptable methods of highly-effective contraception (ie, methods that result in a failure rate of <1% per year) are:

- Combined (estrogen and progestogen containing) hormonal contraceptives associated with inhibition of ovulation (oral, intravaginal, transdermal) stabilized for at least 30 days prior to the screening visit (Visit 1) plus a barrier method (eg, condoms or diaphragms with spermicidal gel or foam)
- Progestogen-only hormonal contraception associated with inhibition of ovulation plus a barrier method
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Male sterilization/vasectomized partner
- True abstinence: When this is in line with the preferred and usual lifestyle of the subject (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods], declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.)

4.4.2 Male Contraception

Contraception is required for all sexually-active male subjects and their partners. All male subjects (including those who are sterile) agree not to donate sperm, and to use 1 of the following approved methods of contraception from the baseline visit on Day 0 until 30 days following study discharge:

- Male condom with spermicide
- Sterile sexual partner
- Intrauterine device with spermicide (use by female sexual partner)
- Female condom with spermicide (use by female sexual partner)
- Contraceptive sponge with spermicide (use by female sexual partner)
- Intravaginal system (eg, vaginal ring with spermicide, a diaphragm with spermicide, or a cervical cap with spermicide) (use by female sexual partner)
- Oral, implantable, transdermal, or injectable hormonal contraceptive (use by female sexual partner) plus a barrier method

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from the IP with the CRO Medical Monitor when possible.

If the IP is discontinued, regardless of the reason, the evaluations listed for both Visit 9/end of study (EOS) and Visit 10/Follow-up, are to be performed as completely as possible; however, the EOS liver biopsy will not be required for patients discontinuing prior to Week 44.

Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination, date of stopping the IP, and the total amount of the IP taken must be recorded in the electronic case report form (eCRF) and source documents.

Subjects who discontinue will not be replaced.

4.5.1 Subject Withdrawal Criteria

Medically important events that in the opinion of the investigator, medical monitor or sponsor would compromise the subject's ability to safely continue in the study may result in withdrawal of the subject from the study. Subjects who become pregnant or demonstrate disease progression, defined as the development of signs or symptoms of hepatic decompensation (ie,

esophageal variceal hemorrhage, hepatic encephalopathy, ascites, or hepatocellular carcinoma), will be withdrawn from the study and followed as set forth in the protocol.

4.5.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the eCRF. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event (AE)
- Protocol violation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Screen failure
- Noncompliance with study drug
- Physician decision
- Pregnancy
- Progressive disease (refer to Section 4.5.1)
- Study terminated by sponsor
- Other - If "Other" is selected, the investigator must specify on the eCRF

4.5.3 Subjects Lost to Follow-up Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact clinic visit. At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

4.5.4 Safety-related Stopping Rules

Refer to Appendix 4 and Appendix 5 for criteria to assess severity of liver-related AEs and stopping rules for liver-related blood tests, respectively.

An urgent safety review will be conducted within 7 days by the sponsor and in consultation with the DMC if 1 or more of the following criteria are met:

- Death that is considered related to the study drug
- Two SAEs of similar type (defined as same or similar Medical Dictionary for Regulatory Activities (MedDRA) higher level group code), and considered related to the study drug.

The urgent review will be performed by a sponsor safety review group, which will include the study Pharmacovigilance and Risk Management (PVRM) Medical Lead and the PVRM therapeutic area (TA) Head, and in consultation with the DMC. The PVRM TA Head, not the PVRM Medical Lead involved in the study, may be unblinded as part of this urgent safety review, if required. Following the sponsor's review of safety data and consultation with the DMC, one of the following actions will be taken with respect to study status:

- Continue study with protocol unchanged
- Continue study with modifications to the protocol
- Terminate study

Subject safety will be monitored on a continuous basis during this study until the last subject completes his or her last scheduled study visit/assessment.

5. PRIOR AND CONCOMITANT TREATMENT

All non-study treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to signing informed consent at the screening visit (Visit 1) and through the final study contact (including protocol-defined follow-up period) must be recorded on the appropriate eCRF page.

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, and behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate), received within 30 days of the date of first dose of the IP. Prior treatment information must be recorded on the appropriate eCRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of the IP and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate eCRF page.

5.2.1 Permitted Treatment

Medications and supplements including but not limited to vitamin E, betaine, s-adenosyl-l-methionine, ursodeoxycholic acid, milk thistle, gemfibrozil, anti-TNF therapies, probiotics biguanides (metformin), thiazolidinediones (TZDs) and GLP-1 RAs that have been used to treat NAFLD/NASH are allowed during the course of the study if the subject has been on a stable dosing regimen (ie, same dose and frequency in the previous 6 months prior to the qualifying liver biopsy and not initiated after qualifying liver biopsy) and will continue this dosing regimen throughout study participation. Use of antiplatelet medications is also allowed. The investigator must contact the CRO medical monitor then the Shire GCDL to discuss any changes to concomitant medications that may impact the study.

5.2.2 Prohibited Treatment

The following list of prohibited drugs cannot have been taken for more than 2 weeks within the year prior to randomization and are excluded while on study.

- Systemic Glucocorticoids
- Tamoxifen
- Amiodarone
- Methotrexate
- Alcohol (see Section 4.2)
- Griseofulvin
- Valproate
- Nucleoside Analogues
- Tetracycline (high dose)
- Estrogens at doses greater than used for hormone replacement
- Anabolic steroids
- Bile acid sequestrants such as

- Total parenteral nutrition

cholestyramine or colestipol

- Any other known hepatotoxins including over-the counter therapies and herbal therapies such as germander, chaparral and ma-huang.

This is not a comprehensive list. Treatments not listed above are generally considered allowable, unless considered a potential hepatotoxin. Antidiarrheals will be allowed at the discretion of the investigator, with the exception of BA sequestrants.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is volixibat potassium (volixibat), which will be provided in 5, 10 and 20 mg capsule form. Additional information is provided in the current Volixibat Potassium (SHP626) Investigator's Brochure.

The reference/comparator product is an identical PBO which will be provided in capsule form.

6.1.1 Blinding the Treatment Assignment

The IP will be supplied as double-blind blister packs. The actual double-blind treatment given to individual subjects is determined by a randomization schedule which will be automatically assigned by the interactive response technology (IRT). Placebo capsules, which exactly match the IP, will be used in the blister packs to provide the same number and size capsules for each of the doses within the treatment groups.

6.2 Administration of Investigational Product(s)

All IP and supplies will be provided by Shire or its designee**.** At each visit, subjects will be supplied with enough IP to last until the subsequent visit. Lost or damaged IP will be replaced as needed. Volixibat will be supplied to the clinical research center (CRC) as powder in capsule. Volixibat will be supplied in identical capsules in strengths of 5, 10, and 20 mg (with matched PBO).

6.2.1 Interactive Response Technology for Investigational Product Management

IRT will be used for the following investigational tasks:

- Randomization
- Supply management
- Inventory management and supply ordering
- Expiration tracking
- Returns
- Emergency unblinding

6.2.2 Allocation of Subjects to Treatment

This is a double-blind, PBO-controlled study. The actual treatment given to individual subjects is determined by a randomization schedule.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation.

The randomization number represents a unique number corresponding to the IP allocated to the subject, once eligibility has been determined.

Individual subject treatment is automatically assigned by the IRT.

Once a randomization number/unique identifier has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. If a randomization number/unique identifier is allocated incorrectly, the clinical research associate (CRA)/study monitor must be notified as soon as the error is discovered.

IP packaging identification numbers, separate from randomization numbers/unique identifiers, may also be assigned to subjects for specific treatment assignment as dictated by the study. In these cases, the same IP packing identification number may not be assigned to more than 1 subject.

Subjects will be equally allocated to all volixibat doses and PBO if their randomization precedes the 24 week IA. If a subject's randomization occurs after the 24 week IA, subjects will be equally allocated to all remaining volixibat doses (determined based on the results of the IA) and PBO. The randomization will be stratified by baseline T2DM and NAS $\geq$6 or NAS = {4, 5}.

6.2.3 Dosing

All doses of volixibat or matching PBO will be administered orally as a capsule in a double-blinded fashion. The first dose of IP for each subject will be administered in the clinic. The dose will be administered with 240 mL of water and should be given 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat. All assessments should be completed at least 30 minutes prior to administration of the IP. The subject should make all attempts to consistently take the IP around the same time each day. If a dose is missed at the normally scheduled time, the subject can make up the dose that day; however, they should not take two doses in any 24-hour period.

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be broken during the study except in emergency situations where the identification of the IP is required for further treatment of the subject. The investigator should contact the CRO medical monitor and the Shire GCDL at the same time and as soon as possible after the investigator has been unblinded.

In the event that the treatment assignment is broken, the date and the signature of the person who broke the code are to be recorded in the source documents and the IRT, and the reason for breaking the code will be recorded in the source documents and the clinical database. Upon

breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code-breaks that occur must be reported to the CRO medical monitor. Code-break information is held by the pharmacist/designated person at the site and by the CRO medical monitor for the study or designee.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled with a minimum of the protocol number, medication identification number, dosage form (including product name and quantity in pack), directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements 'For clinical trial use only', and/or 'CAUTION: New Drug - Limited by Federal (or USA) Law to Investigational Use', 'Keep out of reach of children', and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Additional labels may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The sponsor or designee will provide the IP for this study. The IP is packaged in the following labeled containers:

- Volixibat 5 mg capsules
- Volixibat 10 mg capsules
- Volixibat 20 mg capsules
- Volixibat PBO capsules

6.3.3 Storage

The investigator has overall responsibility for ensuring that the IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented.

IPs are distributed by the pharmacy or nominated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP labels as they are distributed.

All IP must be stored at the clinic at 20 - 25°C (68 - 77°F); excursions are allowed between 15 - 30°C (59 - 86°F).

IPs must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the IP is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion below 15°C (59°F) or above 30°C (86°F); these excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room or a change in storage location.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing the IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed IP will be documented on the eCRFs and/or other IP record. The investigator is responsible to ensure the retrieval of all study supplies from subjects.

No IP stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records. The site must ensure that the accountability and destruction records are complete, accurate, and ready for verification at each monitoring visit.

The process for return and destruction of IP must be determined and documented during the study start-up phase.

With the written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject returned IP, and empty/used IP packaging may be destroyed at the site or a local facility on an ongoing basis throughout the study and at the end of the study following verification of accountability by the CRA/study monitor. In this case, destruction records identifying what was destroyed, when, how, and by whom, must be obtained with copies provided to the sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Alternatively, in the absence of written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject-returned IP, and empty/used IP packaging may be required to be sent to a nominated contractor on behalf of the sponsor for IP destruction on an ongoing basis throughout the study and at the end of the study. IP being returned to the sponsor's designated contractors also must be counted and verified by clinical site personnel and the CRA/study monitor. For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, IRT) do not require a shipment form. Returned IP must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any IP prior to shipment. Shipment of all returned IP must comply with local, state, and national laws.

6.5 Subject Compliance

Subjects must be instructed to bring their unused IP and empty/used IP packaging to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, blister pack) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details on the drug accountability form.

7. STUDY PROCEDURES

7.1 Study Schedule

This study will consist of a screening period of up to 56 days (the screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor), a 48-week Treatment period, and a Follow-up visit 4 weeks after treatment ends. A detailed display of all study procedures is provided in Table 1.

Patients will be assessed according to the following schedule:

- Screening – Visit 1 (Weeks -8 to 0 [Day -56 to Day -1])
- Baseline – Visit 2 (Week 0 [Day 0])
- Treatment and Assessments – Visits 3 through 9 (Week 2 through Week 48)
- Follow-up – Visit 10 (Week 52, 4 weeks after completion of dosing)

7.1.1 Screening (Visit 1)

Screening procedures must be completed within 56 days prior to randomization for the first dose of the IP. The screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor. At the screening visit, considered Visit 1 (Week -8 to 0, Day -56 to -1), all screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from the subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

The following screening procedures should be assessed at the beginning of the visit window, preferably between Day -56 and Day -49: inclusion/exclusion criteria, collection of subject information (demographics, medical and medication history AEs, and concomitant medications), a physical examination including vital signs, height, weight, and waist/hip measurements; collection of blood and urine samples for screening and safety assessments, performance of an electrocardiogram (ECG), and scheduling of MRI and liver biopsy. MRI and liver biopsy (if one has not been completed in the previous 6 months) should be performed with sufficient time to ensure results are received prior to the baseline visit.

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered the IP. Subjects cannot be rescreened once they have been designated as a screen failure. However, a subject's abnormal screening lab results may be repeated for confirmation before designating a subject as a screen failure.

7.1.2 Baseline (Visit 2)

Following the screening visit, subjects will return to the clinic within 56 days for Visit 2, (considered Week 0, Day 0), for baseline assessments including review of the inclusion/exclusion criteria, adverse events, and concomitant medications; physical examination with vital signs, weight, and waist/hip measurements; ECG; collection of blood and urine samples; stool assessment; and completion of the patient-reported outcome (PRO) EuroQol-5 Dimension-5 Level Questionnaire (EQ-5D-5L) survey. After completion of these assessments, subjects are randomized using Interactive Response Technology (IRT) to 1 of 4 treatment arms receiving volixibat 5 mg, 10 mg, or 20 mg, or PBO. The initial supply of study drug is dispensed to ensure adequate daily dosing until the next scheduled study visit.

7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)

During the 48 weeks of double-blind treatment, 7 clinic visits are scheduled to occur as follows:

- Visit 3 - Week 2, Day 14 (+/- 3 days)
- Visit 4 - Week 4, Day 28 (+/- 3 days)
- Visit 5 - Week 8, Day 56 (+/- 5 days)
- Visit 6 - Week 12, Day 84 (+/- 5 days)
- Visit 7 - Week 24, Day 168 (+/- 7 days) – Interim Analysis
- Visit 8 - Week 36, Day 252 (+/- 7 days)
- Visit 9 - Week 48, Day 336 (+/- 7 days) – End of Study

Subjects are reminded not to eat and not to take their study drug on the day of scheduled study visits prior to completion of assessments. They should bring their study drug with them to the visit. All assessments should be completed at least 30 minutes prior to administration of the IP. IP should be taken 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat.

The Schedule of Assessments provided in Table 1 details the procedures to be completed at each visit. All Treatment visits (3 through 9) will include weight and waist/hip measurements, assessment of vital signs, and blood sampling for completion of biochemistry, hematology, serum glucose, lipid panel, HbA1c, and serum liver-related blood tests. At all visits, adverse events, and concomitant medications will be collected for all subjects, and female subjects of childbearing potential will have a urine pregnancy test. Subjects will return containers of unused study drug for assessment of accountability and compliance which will be documented in the IRT. New supplies of study drug will be dispensed at Visits 3 through 8.

Additional assessments will also occur less frequently during the Treatment Period for vitamin A, vitamin D, vitamin E, vitamin K via PT/INR, insulin, thyroid testing, urinalysis, ECG, physical examinations, stool assessment, and completion of the EQ-5D-5L.

An MRI will be repeated at Visit 7 for the 24-week IA and at Visit 9 (Week 48) for all subjects. A final liver biopsy will be performed at Visit 9 (Week 48) for all subjects unless a subject discontinues prior to Week 44, in which case the EOS liver biopsy is not required.

7.1.4 Follow-up (Visit 10)

The follow-up period for this protocol is 4 weeks after the last dose of study drug with a final Follow-up visit scheduled for Week 52. Procedures to be completed at this final visit include physical examination, weight, waist/hip measurements, vital signs, samples for biochemistry and hematology, serum glucose, lipid panel, HbA1c, serum liver-related blood tests, insulin, thyroid testing, stool assessment, and urine pregnancy test (for women of childbearing potential). Adverse events and concomitant medications will be recorded. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.5 Additional Care of Subjects after the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

All assessments and procedures are to be performed by the Investigator or a qualified designee who has been trained in the protocol. Assessments are to be performed according to the schedules shown in Table 1. If a subject terminates the study early, the CRC will make reasonable effort to perform the EOS and safety follow-up assessments and procedures for the subject's safety and well-being.

7.2.1 Demographic and Other Baseline Characteristics

Demographic details will be obtained at screening and recorded on the eCRF. Data collected will include age, gender, ethnicity, height, and weight.

7.2.2 Efficacy

7.2.2.1 Liver Biopsy

Liver biopsies will provide histologic data for confirmation of the diagnosis of NASH, assessment and grading of NASH activity, and scoring of steatosis, lobular inflammation, ballooning, as well as fibrosis and additional features (see Appendix 2 and Laboratory Manual for additional information).

7.2.2.2 MRI

The presence of ≥5% steatosis on screening MRI from a centrally read radiologist will be performed either during the screening period or within 6 months prior to the first visit. Steatosis is assessed by MRI hepatic fat fraction. The Week 24 MRI will be conducted for subjects in the IA set only.

7.2.3 Safety

7.2.3.1 Medical History and Medications

Medical history including important medical events and concomitant medication and illnesses will be obtained at screening and will be recorded on the eCRF. Any existing medical condition present prior to the time of randomization should be reported as medical history

7.2.3.2 Physical Examination

A complete physical examination will be performed with a thorough review of body systems at screening, baseline prior to randomization, and at study visits specified in Table 1. Physical examinations will include a review of the subject's general appearance, as well as evaluation of the body systems including:

- Eyes, ears, nose, throat
- Lymph nodes
- Cardiovascular
- Skin
- Abdomen
- Neurological
- Spine and extremities

Abnormalities identified at the screening visit (Visit 1) will be documented in the subject's source documents and on the medical history eCRF. Changes after the screening visit (Visit 1) will be captured as AEs on the AE eCRF page, if deemed clinically significant by the investigator.

Height will be measured at the screening visit only while weight and waist and hip circumference will be recorded at all study visits. BMI will be calculated programmatically.

7.2.3.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.3.4 Vital Signs

Vital signs include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate. Blood pressure should be determined by cuff (using the same method, the same arm, and in

the same position throughout the study). Any deviations from baseline (Visit 2) vital signs which are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

7.2.3.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the central laboratory's normal procedures. Reference ranges supplied by the central laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Clinically significant findings should be evaluated for recording as adverse events on the eCRF. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Blood urea nitrogen (BUN)
- C4
- Calcium (Ca)
- Bicarbonate (CO2)
- Chloride (Cl)
- Creatinine
- Creatine kinase
- Gamma glutamyl transferase (GGT)
- Glucose
- Lactate dehydrogenase (LDH)
- Magnesium (Mg)
- Phosphate (P)
- Potassium (K)
- Sodium (Na)
- Total and direct bilirubin
- Total cholesterol
- Protein
- Triiodothyronine (T3)
- Thyroid-stimulating hormone (TSH)
- Triglycerides
- Uric acid

Hematology

- Hemoglobin
- Hematocrit
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular volume (MCV)
- Platelets
- Red blood cell (RBC)
- White blood cell (WBC) count with differential
- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Urinalysis

- Appearance (clarity and color)
- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopic examination of sediment
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen
- Oxalate

7.2.3.6 Additional Laboratory Assessments

Laboratory samples will be collected and assessed for:

- HIV testing and assessment of Hepatitis B/C including HBcAb, HBsAg, HBVDNA, and HCVAb, and HCVRNA will occur at the screening visit only
- Lipid panel including fasting total cholesterol, HDL-C, LDL-C, and triglycerides at each scheduled study visit (Visits 1 through 10)
- HbA1c will be tested at every scheduled visit (Visits 1 through 10)
- Full coagulation panel will be done at screening and baseline, but only PT/INR is required at remaining time points at Visits 4, 7, and 9
- Insulin will be tested at screening, baseline, at Visit 4 and Visits 6 through 10
- HOMA-IR and HOMA-%B: will be calculated programmatically from serum glucose tested at screening, baseline, Visits 7, 9, and 10

- Vitamin A, Vitamin D, Vitamin E, and Vitamin K (via PT/INR) will be tested at screening, baseline, and Visits 4, 7, and 9

- Thyroid testing including TSH and T3 will be tested at screening, baseline, and Visits 4, 6, 7, 9, and 10

- C4 samples will be collected at baseline and Visits 6, 7, 9, and 10

7.2.3.7 Pregnancy Test

A urine pregnancy test is performed on all females of child-bearing potential at the screening visit (Visit 1), baseline visit (Visit 2), at each Treatment visit (Visits 3 through 9) and at the Final visit (Visit 10), or if pregnancy is suspected, or on withdrawal of the subject from the study. A positive urine pregnancy test must be followed with a serum pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Section 8.1.7.

7.2.3.8 Drug and Alcohol Testing

A urine screen for drugs of abuse and blood test for alcohol will be performed at screening and baseline as described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the eCRF database.

Any positive result for drugs of abuse at the screening or baseline visits may exclude the subject from further participation in the study. A positive test for drugs of abuse or alcohol done at the investigator's discretion discovered at any time during the study will be grounds for study discontinuation.

7.2.3.9 Electrocardiogram

An ECG (12-lead) will be performed at the times specified in Table 1 in accordance with the clinical site's standard practice(s) and equipment supplied by the CRC. Recordings of ECGs will be read locally at the clinical site by the investigator or designee. The ECG will include assessments of heart rate and PR, RR, QRS, and QT intervals. Identification of any clinically significant findings and/or abnormalities will be recorded on the eCRF.

7.2.3.10 Health-related Quality of Life Assessments

The EuroQol (EuroQol. 2016. Available at: http://www.euroqol.org. [Accessed 17 February 2016]) EQ-5D-5L ("EQ-5D") is a widely used standardized questionnaire that assesses generic HRQoL and is also recommended for health-economic evaluations. The EQ-5D includes two components: a descriptive profile and a visual analogue scale (VAS). The descriptive profile

includes five dimensions (ie, pain/discomfort, mobility, usual activities, self-care and anxiety/depression), each with five levels (ie, no problems, slight problems, moderate problems, severe problems, extreme problems). An EQ-5D index can also be derived from the data which summarizes health status using a single value (ie, health-state utility). The psychometric properties of the EQ-5D-5L have been established and well documented (see Appendix 3).

7.2.3.11 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 146 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 146 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not the IP is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE eCRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of the IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of the IP, but the dyspepsia becomes severe and more frequent after first dose of the IP has been administered, a new AE of severe dyspepsia (with the appropriate date of onset) is recorded on the appropriate eCRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Appendix 4 provides criteria to assess the severity of liver-related adverse events.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to the IP for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the IP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the eCRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown.

8.1.4 Symptoms of the Disease Under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation of the disease and are part of the efficacy data to be collected in the study; however, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pre-treatment value observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.6 Criteria for Discontinuation of Treatment

Appendix 5 provides guidelines for discontinuation of treatment based on elevated ALT, AST, TB, and associated signs and symptoms.

8.1.7 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.4.

Any report of pregnancy for any female study participant (or the female partner of a male participant) must be reported within 24 hours to the Shire Global Pharmacovigilance and Risk Management Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO medical monitor and the Shire clinical physician using the details specified in the Emergency Contact Information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-HCG test or ultrasound result will determine the pregnancy onset date.

8.1.8 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of the IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- Misuse – Intentional use of the IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol)

- Overdose – Intentional or unintentional intake of a dose of an IP exceeding a pre-specified total daily dose of the product.

- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the Volixibat Potassium (SHP626) Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Pharmacovigilance and Risk Management Department and the Shire GCDL within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.8) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Pharmacovigilance and Risk Management Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the Shire GCDL using the details specified in the Emergency Contact Information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence (whether considered to be related to the IP or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect

- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. 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.

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.4, and must be reported to the Shire Global Pharmacovigilance and Risk Management Department and the Shire GCDL within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Pharmacovigilance and Risk Management Department within 24 hours of the first awareness of the event.

8.2.4 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.5 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received the IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

8.2.6 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The CRO is responsible for notifying the relevant regulatory authorities as appropriate: USA central IRBs/EU central ECs of related, unexpected SAEs.

In addition the CRO is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP626-201 program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the eCRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Data that may potentially unblind the treatment assignment (ie, IP serum concentrations, antibiotics to investigation product, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513).

9.5 Planned Interim Analysis and Data Monitoring Committee

9.5.1 Planned Interim Analysis

Study enrollment will be paused after 92 subjects have been randomized in order for an IA to be conducted after at least 80 subjects have received 24 weeks of treatment. To protect study integrity, the IA will be performed by an independent statistician and statistical reporting group who are not involved in the clinical trial conduct and are not responsible for the final analysis of clinical trial data. The IA will be performed to evaluate the safety, tolerability, and efficacy of 3 doses of volixibat each compared to PBO and be used to drop 1 or more doses of volixibat, or to terminate the trial.

The criteria for deciding whether or not to continue randomizing to a particular volixibat dose following the Week 24 IA will be specified in the DMC charter. In general, randomization to any volixibat dose determined to be unsafe, intolerable or ineffective/futile at the IA will be discontinued. If all of the volixibat doses are determined to be intolerable or ineffective/futile the clinical trial will be terminated. If none of the volixibat doses are determined to be unsafe, intolerable or ineffective/futile then randomization to the volixibat dose that is the least promising or least helpful in characterizing the dose-response relationship (as specified in the DMC charter) will be discontinued. Tolerability will be assessed by comparing the number of discontinuations due to any one TEAE (presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain) between each volixibat dose group and PBO group. Efficacy will be assessed based on a reduction of steatosis (assessed by MRI-HFF) and ALT by comparing the change from baseline in MRI-HFF and percent change from baseline in ALT at Week 24 between each volixibat dose group and PBO group.

Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. That is, a subject receiving a dose to which randomization is stopped after the IA will continue to receive that same dose throughout the course of the study, and complete all required assessments, unless a dose is determined to be unsafe, in which case the affected subjects would be discontinued from the study and followed as per Section 4.5. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining volixibat dose groups or PBO group by IRT. Subjects will be randomized to 1 or 2 volixibat doses or to PBO. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of IP.

9.5.2 Data Monitoring Committee

An independent DMC will be established to assess safety, tolerability, and efficacy during the study, as well as to ensure the validity and scientific merit of the trial. The DMC will monitor ongoing data generated by the study at regular intervals for the duration of the study. Their role

is to protect the interests of the subjects in the study and of those still to be entered, by review of accumulating data generated in the study. If a safety concern is identified (eg, if either of the criteria are met in Section 4.5.4, Safety-related Stopping Rules), the DMC may recommend an action to the sponsor on further study conduct, including stopping the study at any time.

In addition, the DMC will review the results of the Week 24 IA and make recommendations concerning study discontinuation due to intolerability or futility, or the continued randomization of 1 or more volixibat dose groups and PBO.

The roles, responsibilities, and rules governing operation of the DMC will be discussed in full in a DMC charter. The DMC charter will define the primary responsibilities of the DMC; guide its activities, its relationship with other study components, its membership, and the purpose and timings of its meetings. It will provide the procedures for ensuring confidentiality, formal communication, and outline of the content of reports that will be provided by the DMC.

Appropriate summary statistics and data listings will be provided to the DMC by an independent statistician supported by an independent statistical reporting group not otherwise assigned to the study.

The recommendations made by the DMC to alter the conduct of the study or to stop the study will be forwarded to Shire for final decision. The implementation of any DMC recommendation is solely the responsibility of the sponsor. Shire will forward such decisions to regulatory authorities, as appropriate.

9.6 Sample Size Calculation and Power Considerations

These are the primary hypotheses that are being tested in this study:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does not differ between any of the volixibat doses and PBO.

- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS does differ between at least one volixibat dose and PBO.

Response rates of 21% (PBO) and 45% (active) were reported in the FLINT trial (Neuschwander-Tetri et al. 2015) using the same primary end-point of reduction of NAS by at least two points (without worsening of fibrosis). Based on those response rates, 67 subjects per group completing the trial are needed for 80% power with a 10% type I error for a study comparing two volixibat doses to PBO. Holm multiplicity adjustment was included in the sample size estimate.

Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 PBO) a 24-week IA will eliminate at least one dose group based on the criteria provided to the DMC; enrollment will

continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 active and 1 PBO) continue. Fewer subjects are needed if more than one active dose is dropped.

9.7 Study Population

The Screened Set will consist of all subjects who have signed an informed consent.

The Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-baseline safety assessment (eg, coming back for any visit, reporting of an AE or reporting the absence of AEs).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, liver biopsy, serum liver-related biochemistry measurement).

The Interim Analysis Set (IAS) will consist of all subjects in the Safety Analysis Set who have at least 1 post-baseline efficacy assessment (eg, MRI, ALT biochemistry measurement) on or before the Week 24 visit at the data cut time of the IA.

9.8 Efficacy Analyses

All efficacy analyses will be based on the FAS and all statistical tests will be 2-sided hypothesis tests performed at the 10% level of significance. Also, all confidence intervals will be 2-sided confidence intervals, unless otherwise stated.

9.8.1 Primary Efficacy Endpoint

The primary endpoint is the binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points, without worsening of fibrosis, from baseline NAS.

The FAS will be used to assess the primary efficacy endpoint. The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS will be analyzed. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of T2DM at baseline and baseline NAS separated into two groups (NAS={4, 5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level.

Analyses which explore the impact of the missing data on the primary efficacy endpoint will be conducted. These analyses may compare imputations of the missing values which favor the PBO and/or imputations which favor the active. In addition, analyses utilizing existing HFF and/or ALT data may be performed. Further, if the pattern of missing values does not appear uniformly distributed among the treatment arms, imputation method(s) based on informative missingness

may also be performed. Analyses investigating the impact of missing data will be detailed in the Statistical Analysis Plan.

9.8.2 Secondary Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the secondary efficacy endpoints. Binary secondary efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous secondary efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
- Change from baseline to Week 48 on hepatic steatosis as measured by MRI-HFF.
- Change from baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH CRN)
- Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at Week 48.
- Change from baseline to Week 48 on serum liver-related biochemistry as measured by:
    - alanine aminotransferase (ALT)
    - aspartate aminotransferase (AST)
    - alkaline phosphatase (ALP)
    - gamma glutamyl transferase (GGT)
    - total bilirubin (TB)
- Change from baseline to Week 48 on metabolic indicators as measured by:
    - fasting serum glucose levels
    - insulin levels
    - HbA1c
- Change from baseline to Week 48 on serum lipids measured by:
    - fasting total cholesterol

- HDL-C
- LDL-C
- triglycerides

9.8.3 Exploratory Efficacy Endpoints

Descriptive statistics will be presented for each time point at which the variable is measured (see Table 1) for the exploratory efficacy endpoints. Binary exploratory efficacy endpoints will be analyzed with the same method (stratified Cochran-Mantel-Haenzel test) as the primary efficacy endpoint. Continuous exploratory efficacy endpoints will be analyzed with an ANCOVA model with change from baseline as the outcome variable; treatment group, presence or absence of T2DM at baseline, and baseline NAS separated into 2 groups (NAS={4,5} or NAS={6,7,8}) as factors and baseline values as a covariate. Endpoints include:

- Change from baseline to Week 48 on liver histology as measured by the SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F)
- Change from baseline to Week 48 on anthropomorphic measures:
  - body weight
  - BMI
  - waist circumference
  - waist-hip ratio
- Change from baseline to Week 48 in subjects with T2DM on homeostasis measured by:
  - Homeostasis model assessment -IR (HOMA-IR)
  - HOMA-beta cell function (HOMA-%B)

9.9 Safety Analyses

The Safety Analysis Set will be used to assess the safety endpoints.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. Treatment-emergent AEs are defined as AEs that start or worsen on or after the date of the first dose of IP, and no later than the follow-up visit. The number of events, incidence, and percentage of TEAEs will be calculated by SOC, by preferred term, and by treatment group. Treatment-emergent AEs will be further summarized by severity and relationship to the IP. Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized by treatment group and visit. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

9.10 Other Analyses

9.10.1 Health-related Quality of Life Analyses

- Change from baseline to Week 48 on the EQ-5D index (Appendix 3). The EQ-5D index will be derived using published UK-based preference weights. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from baseline to Week 48 on the EQ-5D VAS score. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from baseline to Week 48 in the proportion of subjects reporting having problems (none, slight, moderate, severe, extreme) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire. Proportions and 95% confidence intervals will also be generated for the baseline and Week 48 values as well as for change from baseline.

9.10.2 Stool Assessment

Stool hardness and number of evacuations will be assessed throughout the study. Stool hardness will be assessed for the softest evacuation within 24 hours of each clinic visit using the Bristol Stool Chart, a medical aid designed to classify the form of human feces into 7 categories where Type 1 is the hardest and Type 7 is the softest. Appendix 6 provides a sample of the Bristol Stool Chart. Number of evacuations within the past 24 hours prior to the clinic visit will be recorded at specified times as per Table 1. Descriptive statistics by treatment group will be presented for each time point at which the variable is measured.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements.

The name and address of each third party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of the IP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

10.1.3 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the

Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

10.1.5 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.do

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all the IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

eCRFs are supplied by the sponsor and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. eCRFs must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

All eCRF data must be endorsed by the investigator.

The CRA/study monitor will verify the contents of the eCRF against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, subject diary cards, original clinical laboratory reports, and histology and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local

regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, images, etc.). Non-study site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other USA national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in the IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB/EC and which received their favorable opinion/approval. A copy of the

IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor, prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an EC opinion can be the sponsor, the investigator, or for multicenter studies the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the sponsor or its designee has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor, the investigator or for multicenter studies the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All USA-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the sponsor or its designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or

market volixibat; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results / Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee, which is done for large, multicenter Phase 2-4 and certain other studies as determined by Shire. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written

agreement to publish, and shall be given to the sponsor for review at least 60 days prior to submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

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12. APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	24 February 2016	Global
Amendment 1	8 April 2016	Global
Amendment 2	19 July 2016	Global

APPENDIX 2 FATTY LIVER DISEASE HISTOLOGY SCORING

Diagnosis: NASH, Suspicious/Borderline NASH (type a or b), NAFLD, Not NAFLD

NAS SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Lobular Inflammation	0	no foci
		1	< 2 foci per 200x field
		2	2-4 foci per 200x field
		3	> 4 foci per 200 x field
	Ballooning	0	None
		1	Rare or diagnostically borderline
		2	Many or Prominent ballooned hepatocytes

SAF SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Ballooning	0	normal hepatocytes with cuboidal shape and pink cytoplasm
		1	clusters of hepatocytes with rounded shape and pale cytoplasm usually reticulated
		2	grade 1 plus enlarged hepatocytes, 2 x bigger than normal cells.
	Lobular Inflammation	0	none
		1	< 2 foci per 200x field
		2	>2 foci per 200x field

Steatosis 1,2,3 + Ballooning 1,2 + Lobular Inflammation 1,2 = NASH
Steatosis 1,2,3 +Ballooning 0 + Lobular Inflammation 0,1,2 = NAFLD
Steatosis 1,2,3 + Ballooning 1,2 + Lobular 0 = NAFLD
Steatosis 0 = no NAFLD

FIBROSIS SCORE	None	0
	Mild zone 3 perisinusoidal (requires trichrome)	1a
	Moderate Zone 3 perisinusoidal (visible on H&E)	1b
	Portal/periportal only	1c
	Portal, periportal and perisinusoidal	2
	Bridging	3
	Cirrhosis	4

References: Kleiner et al. 2005; Bedossa 2012

ADDITIONAL SCORING FEATURES

Expanded Balloon Score

0: None

1: Few Non classic

2: Few Classic

3. Many Classic

4: Severe Classic

Portal Inflammation

0: None

1: Minimal

2: Mild

3: More than Mild

Megamitochondria

0: None

1: Present

Acidophil Bodies

0: None or rare

1: Present

For stage 2 or greater fibrosis

Portal Predominant

Central Predominant

No predominance

Steatosis Zone

Zone 1 predominant

Zone 3 predominant

Azonal

Panacinar

Glycogenosis

None

Focal

Diffuse

APPENDIX 3 EQ-5D-5L HEALTH QUESTIONNAIRE UK SAMPLE ONLY, NOT FOR OFFICIAL USE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

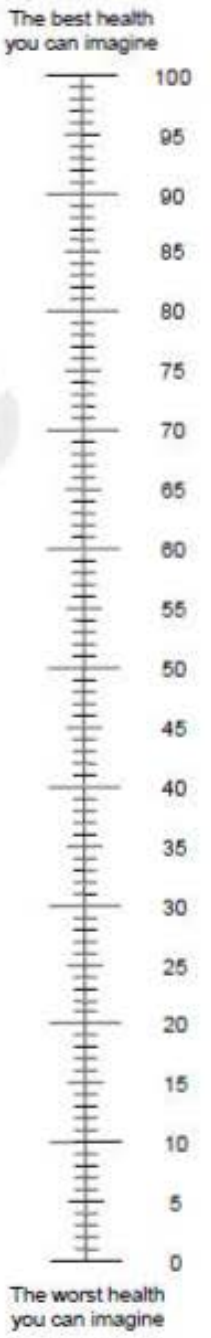
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 4 CRITERIA TO ASSESS SEVERITY OF LIVER-RELATED ADVERSE EVENTS

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
ALT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
AST	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Alkaline Phosphatase	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
GGT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Bilirubin	Normal	>1.0-1.5	>1.5-2.5	>2.5-5	>5

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
NOTE: Values expressed as multiples of the upper limit of the normal range (ULN).

APPENDIX 5 STOPPING RULES FOR LIVER-RELATED BLOOD TESTS

- ALT or AST >6xULN (confirmed with repeat within 24hrs)
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN **and** (TBL >2xULN **or** INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

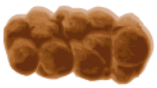
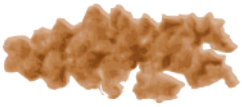
If ALT or AST elevations do not fall within the above parameters, but are > 4 ×ULN subject should remain on study drug with close observation which includes:

1. Immediately contacting the CRO medical monitor.
2. Repeating liver enzyme and serum bilirubin tests two or three times weekly.

   Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
3. Obtaining a more detailed history of symptoms and prior or concurrent diseases.
4. Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
5. Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease., or other disease at the discretion of the investigator
6. Obtaining a history of exposure to environmental chemical agents.
7. Obtaining additional tests to evaluate liver function, as appropriate (eg, INR, direct bilirubin).

APPENDIX 6 BRISTOL STOOL CHART

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

PROTOCOL: SHP626-201

TITLE:	A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)
DRUG:	Volixibat potassium (SHP626)
IND:	123,847
EUDRACT NO.:	2016-000203-82
SPONSOR:	Shire Human Genetic Therapies, Inc. 300 Shire Way Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Philip Newsome MD Director of Centre for Liver Research University of Birmingham Edgbaston, Birmingham B15 2TT UK
NATIONAL/ COORDINATING INVESTIGATOR/UK	Guruprasad P Aithal MBBS, MD, FRCP, PhD Head of the Division, Nottingham Digestive Diseases Centre Director, NIHR Nottingham Digestive Diseases Biomedical Research Unit Nottingham University Hospitals and University of Nottingham Queen's Medical Centre Nottingham NG7 2UH
PROTOCOL HISTORY:	Amendment 1: 8 April 2016 Original Protocol: 24 February 2016

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature: MD FAASLD **Date:** 13 April 2016

Melissa Palmer, MD FAASLD
Global Clinical Development Lead(GCDL)- Hepatology

Investigator's Acknowledgement

I have read this protocol for Shire Study SHP626-201.

Title: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address:
(please hand print or type)

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS VERSION

Protocol Amendments		
Summary of Change(s) Since Last Version of Approved Protocol		
Amendment Number **1**	**Amendment Date** **8 April 2016**	**Global/Country/Site Specific** **Global**
Description of Change		**Section(s) Affected by Change**
Emergency Contact Information updated		Emergency Contact Information
Abbreviations list updated		Abbreviations
Requirement for meal fat content of 10-20 grams before daily dose of investigational product added		Synopsis Table 1 Schedule of Assessments Sections 6.2.3, 7.1.3
Inclusion Criterion #6 clarified fibrosis stage F1-F3		Synopsis Section 4.1
Exclusion Criterion #6 shortened sentence for clarity		Synopsis Section 4.2
Exclusion Criterion #7 cross referenced to Section 5.2.1 Permitted Treatment		Synopsis Section 4.2
Exclusion Criterion #9 referred to Section 5.2.2 Prohibited Treatment		Synopsis Section 4.2
Exclusion Criterion #17 dose for current use of low-dose aspirin (81 mg) allowed		Synopsis Section 4.2
Exclusion Criterion #21 clarified weekly alcohol intake measurement from units to grams/day; added statement that subjects could be excluded at any time during the study based on investigator discretion due to alcohol or substance abuse		Synopsis Section 4.2
Planned duration of screening period increased from 42 to 56 days		Synopsis Table 1 Schedule of Assessments Sections 7.1, 7.1.1, 7.1.2
Secondary endpoint of MRI-HFF clarified for subjects included in the IA		Synopsis
Primary hypothesis was clarified to Null and Alternative hypotheses		Synopsis Section 9.6
Clarified that NAS ≥ 6 is 6, 7, 8		Synopsis Sections 6.2.2
Screening period changed from -6 to -8 weeks		Table 1 Schedule of Assessments Section 3.1 Figure 1 Sections 7.1, 7.1.1
Footnote deleted for ECG; all subsequent footnotes revised		Table 1 Schedule of Assessments
Footnote l revised serum pregnancy test from reflex to serum β-HCG		Table 1 Schedule of Assessments
Reference to Karpen oral presentation at AASLD added		Sections 1.2, 1.3

Inclusion Criterion #4 added cross reference to Section 4.4 Reproductive Potential	Section 4.1
Exclusion Criteria #9 and #10 added cross reference to Section 5.2.2 Prohibited Treatment	Section 4.2
Exclusion Criterion #21 added cross reference to Section 7.2.3.8 Drug and Alcohol Screening	Section 4.2
Acceptable methods of contraception were modified to delete the use of double-barrier methods and clarify barrier methods, add male sterilization, add and define abstinence	Section 4.4.1 Section 4.4.2
Deleted statement regarding withdrawal of subjects for missing >3 consecutive days of dosing	Section 4.5
Redefined PVRM physician as PVRM Medical Lead	Section 4.5.4
Additional permitted medications and supplements were further defined and sponsor medical monitor was redefined as Shire GCDL	Section 5.2.1
Additional prohibited over-the-counter and herbal therapies were specified and a note was added that the list is not comprehensive	Section 5.2.2
Dosing statement deleted from Section 6.2 and moved to Section 6.2.3	Sections 6.2, 6.2.3
Clarified that NAS ≥ 6 is 6, 7, 8; text modified to specify randomization after the Week 24 IA would be equally allocated to all remaining doses.	Section 6.2.2
Text deleted “For blinded studies, there will be a provision for unblinding to ensure adequate treatment of the subject in the case of an emergency.”	Section 6.2.4
Process for drug return or destruction was further clarified	Section 6.4
Added that the screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor.	Sections 7.1, 7.1.1
Clarified that the Week 24 and Week 48 MRIs will be conducted for subjects in the IA set only	Section 7.2.2.2
Serum liver-related tests deleted from list of Additional Laboratory Assessment; calculation of HOMA1-IR and HOMA2-%B added	Section 7.2.3.6
ECG section modified	Section 7.2.3.9
Text related to paper capture (rather than electronic) was deleted	Section 10.2.3.1
Minor typographic and editing corrections where made	Throughout amendment

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious Adverse Events (AEs) Required by the Protocol Form within 24 hours to:

Shire Global Pharmacovigilance and Risk Management Department

Preferred method: scan and e-mail to globalpharmacovigilance@shire.com
OR fax to +44(0) 1256 89 4715 (Global); or +1 866 557 4473 (North America)

AND

A copy of this form must also be sent to the Shire GCDL by fax or e-mail using the details below:

Melissa Palmer, MD, FAASLD, GCDL-Hepatology

fax number: +1 781 482 1822

e-mail: mpalmer@shire.com

For protocol- or safety-related issues during normal business hours 9:00 a.m. to 5:00 p.m. (Eastern Time), the investigator must contact the Contract Research Organization (CRO) Medical Monitor:

ICON Medical Monitor: Anthony Japour, MD

Telephone number: +1 215 616 6439
Fax number: +1 215 616 3096
Mobile number: +1 267 429 6601
E-mail address: Anthony.Japour@iconplc.com

For protocol- or safety-related issues outside of normal business hours, the investigator must contact ICON's 24/7 Medical Emergency Coverage:

Chargeable global telephone number: +1 919 674 5468

NOTE: Investigative sites will be provided country-specific toll-free telephone numbers. Please refer to this document, as applicable. Countries without a toll-free number will need to dial the chargeable number noted above.

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

Please use the information below as applicable to report the Product Quality Complaint:

Origin of Product Quality Complaint	**E-mail Address**
North and South America	PQC@shire.com
European Union and Rest of World	PQCROW@shire.com

Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
1-888-300-6414 or 1-800-828-2088

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ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPPT	activated partial thromboplastin time
ASBT	apical sodium-dependent bile acid transporter
ASBTi	ASBT inhibitor
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BA	bile acid
BMI	body mass index
C4	7-alpha-hydroxy-4-cholesten-3-one
CRA	clinical research associate
CRC	clinical research center
CRO	contract research organization
DMC	data monitoring committee
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimension-5 Level Questionnaire
ECG	electrocardiogram
EOS	end of study
EU	European Union
FAS	full analysis set
FBA	fecal bile acid
FDA	Food and Drug Administration
FXR	farnesoid X receptor
GCDL	Global Clinical Development Lead
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP1 RA	glucagon-like peptide-1 receptors agonists
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBVDNA	hepatitis B virus deoxyribonucleic acid
HCV	hepatitis C virus

HCVAb	hepatitis C virus antibody
HCVRNA	hepatitis C virus ribonucleic acid
HDL-C	high-density lipoprotein-cholesterol
HFD	high fat diet
HFF	hepatic fat fraction
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HOMA2-%B	homeostatic model assessment in β-cell function
HOMA2-IR	homeostatic model assessment insulin resistance
HRQoL	health-related quality of life
IA	interim analysis
IAS	interim analysis set
ICH	International Conference on Harmonisation
IND	investigational new drug
INR	international normalized ratio
IP	investigational product
IR	insulin resistance
IRB	Institutional Review Board
IRT	interactive response technology
LDL-C	low-density lipoprotein-cholesterol
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MedDRA	Medical Dictionary for Regulatory Activities
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MOA	mechanism of action
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NOAEL	no observed adverse effect level
PBC	primary biliary cirrhosis
PBO	placebo
PDFF	proton density fat-fraction
PO	by mouth

POC	proof of concept
PRO	patient-reported outcome
PSC	primary sclerosing cholangitis
PTT	prothrombin time
PVRM	Pharmacovigilance and Risk Management
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAF	steatosis, activity, and fibrosis score
SAP	statistical analysis plan
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TA	therapeutic area
TB	total bilirubin
TEAE	treatment-emergent adverse event
TGR5	transmembrane G protein-coupled receptor
TPN	total parenteral nutrition
TSH	thyroid-stimulating hormone
TZD	thiazolidinediones
ULN	upper limit of normal
USA	United States of America
VAS	visual analogue scale

STUDY SYNOPSIS

Protocol number: SHP626-201	**Drug:** Volixibat potassium (SHP626)
Title of the study: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)	
Number of subjects (total and for each treatment arm): Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 placebo [PBO]) a 24-week interim analysis (IA) will eliminate at least one dose group based on the criteria provided to the Data Monitoring Committee; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue. Fewer subjects are needed if more than one SHP626 dose is dropped.	
Investigator(s): Multicenter study	
Site(s) and Region(s): Anticipated regions -US, Canada, and EU Estimated number of sites planned – 60 to 80	
Study period (planned): July 2016 to July 2019	**Clinical phase:** 2

Objectives:

Primary: To evaluate the effect of volixibat compared to PBO on liver histology

Secondary:

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual nonalcoholic fatty liver disease (NAFLD) activity score (NAS) components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

Rationale: Currently, there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its mechanism of action (MOA) and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

Investigational product, dose, and mode of administration:

- Volixibat 5, 10, and 20 mg and matched PBO capsules by mouth (PO) once daily (QD). Investigational product (IP) should be given 30 minutes prior to the first meal of the day containing approximately 10-20

grams of fat Also see Section 6.2.3 (Dosing).

- Identical PBO will be used as comparator
- Subjects should take the IP at the same time each day and should not take more than one dose in a day if they miss a dose

Methodology:

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

Inclusion and exclusion criteria:

Inclusion Criteria:

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally-authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first Screening visit.
4. Male, or non-pregnant, non-lactating female who is sexually active and who agrees to comply with any applicable contraceptive requirements of the protocol or females of non-childbearing potential.

   Males and females of child-bearing potential who are sexually active, or are not currently sexually active during the period of the study, but become sexually active during the study and 30 days following the last dose of the IP, must agree to use acceptable contraception.
5. Presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F1-F3) from a centrally read liver biopsy performed either during the Screening period or within 6 months prior to the first visit with a NAS of ≥ 4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

Exclusion Criteria:

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV (defined as HCVAb positive without a current or prior history of a detectable HCVRNA) will be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the IP (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.

4. Weight change ≥ 5% after qualifying liver biopsy performed.
5. Contraindications to MRI (ie, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thiazolidinediones (TZD), or glucagon-like peptide-1 receptors agonists (GLP1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation. Also refer to Section 5.2.1, Permitted Treatment.
8. Uncontrolled diabetes defined as HbA1c of ≥ 9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of Screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to Screening. Also refer to Section 5.2.2, Prohibited Treatment.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to qualifying liver biopsy, or initiated any time after qualifying liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST > 5 times upper limit of normal (ULN) at Screening.
12. Serum ALT > 5 times ULN at Screening.
13. Elevated serum creatinine ≥ 2.0 mg/dL.
14. International normalized ratio (INR) >1.3.
15. Total bilirubin (TB) ≥ 2.0 x ULN at Screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count < 130×10^9L
17. Medical history of impaired hemostasis or current use of anticoagulant medication (low-dose, ie 81mg, aspirin (ASA) will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
25. Known positivity for human immunodeficiency virus (HIV) infection.

26. Cancer within 5 years of Screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
27. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
28. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
29. Subject has failed Screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
30. Subjects who are employees at the investigational site.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 56 days
- Planned duration of enrollment period: 364 days
- Planned duration of treatment period: 336 days
- Planned duration of follow-up: 28 days

Endpoints and statistical analysis:

Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-Baseline safety assessment (eg, coming back for any visit, reporting of an adverse event (AE) or reporting the absence of AEs).

Full Analysis Set (FAS) will consist of all subjects in the Safety Set who have a valid scored liver biopsy.

Interim Analysis Set (IAS) will consist of all subjects in the Safety Set who have a valid post-baseline hepatic fat fraction (HFF) assessed by MRI and a valid post-baseline ALT at Week 24.

Screened Set will consist of all subjects who have signed an informed consent.

Endpoints:

- ***Primary:*** Binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS.
- ***Secondary:***
    - Change from Baseline to Week 48 in safety and tolerability of volixibat as measured by the number of subjects discontinuing treatment due to any one treatment-emergent adverse event (TEAE).
    - Change from Baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
    - Change from Baseline to Week 48 on hepatic steatosis as measured by MRI-HFF for subjects included in the IA.
    - Change from Baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH Clinical Research Network (CRN)
    - Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at week 48.
    - Change from Baseline to Week 48 on serum liver-related biochemistry as measured by:
        - alanine aminotransferase (ALT)
        - aspartate aminotransferase (AST)
        - alkaline phosphatase (ALP)
        - gamma glutamyl transferase (GGT)
        - total bilirubin (TB)
    - Change from Baseline to Week 48 on metabolic indicators as measured by

- fasting serum glucose levels
- insulin levels
- HbA1c

- Change from Baseline to Week 48 on serum lipids measured by
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

Primary hypotheses:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS does not differ between any of the volixbat doses and PBO.
- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS does differ between at least one volixbat dose and PBO.

The FAS will be used to assess the primary efficacy endpoint. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of Type 2 diabetes mellitus (T2DM) at Baseline, and baseline NAS separated into two groups (NAS={4,5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level. Histology will be read by one central hepatohistopathologist who will use the NASH CRN standard scoring system – NAS. The SAF Steatosis (S), Activity (A), and Fibrosis (F) scoring system will be determined for exploratory purposes.

The Safety Set will be used to assess the safety endpoints including AEs (including changes from Baseline in physical examination findings), vital signs, ECGs, and clinical laboratory tests (chemistry, hematology, coagulation and urinalysis).

AEs will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as AEs that started or worsened on or after the date of the dose of IP, and no later than the study completion visit (or follow-up visit for subjects who terminate the study early). The number of events, incidence, and percentage of TEAEs will be presented by system organ class and preferred term. TEAEs will be further summarized by severity and the relationship to the IP. AEs related to the IP, AEs leading to withdrawal, SAEs, and death will be listed. The number of discontinuations due to any 1 TEAE will be summarized for each treatment group.

Vital signs, ECG findings, and clinical laboratory tests will be listed for each subject and summarized for each treatment arm, including a flag for any potentially clinically important findings. Graphical presentation may be used when deemed necessary.

For safety parameters, baseline is defined as the last assessment prior to the first dose of the IP.

Planned Interim Analysis:

An IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO, on reduction of steatosis as measured by HFF by MRI and ALT.

Tolerability will be assessed by the number of discontinuations due to any one TEAE, presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Efficacy at the IA will be based on reduction of steatosis or ALT. Steatosis is assessed by MRI-HFF. Depending on the results from the IA, one or more doses of volixibat may be discontinued or the study may be terminated. If the study is not terminated, subjects will receive a total of 48 weeks of the IP.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Informed consent	X									
Inclusion/exclusion criteria	X	X (review)								
Demography, medical & medication history	X									
Physical examination	X	X					X		X	X
Height[c], weight[d], waist circumference and waist:hip ratio	X	X	X	X	X	X	X	X	X	X
Vital signs[e]	X	X	X	X	X	X	X	X	X	X
PRO (EQ-5D-5L)		X					X		X	
Liver biopsy[f]	X								X	
MRI[g]	X						X[h]		X[h]	
ECG (12-lead)	X	X				X			X	
Biochemistry and Hematology[i]	X	X	X	X	X	X	X	X	X	X
Serum Glucose [i,j]	X	X	X	X	X	X	X	X	X	X
Urinalysis[k]	X	X							X	
Urine Drug and Serum Alcohol Tests	X	X								
Urine Pregnancy Test[l]	X	X	X	X	X	X	X	X	X	X
Lipid Panel[i,m]	X	X	X	X	X	X	X	X	X	X
Coagulation Panel[i,n]	X	X		X			X		X	
Vitamin D [i]	X	X		X			X		X	

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-8 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-56 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
HbA1c[i]	X	X	X	X	X	X	X	X	X	X
Serum Liver-Related Blood Tests[i,o]	X	X	X	X	X	X	X	X	X	X
Insulin[i]	X	X		X		X	X	X	X	X
HIV, Hepatitis B/C[i,p]	X									
Thyroid testing[i,q]	X	X		X		X	X		X	X
C4 Sampling		X				X	X		X	X
IRT Accessed	X	X	X	X	X	X	X	X	X	X
Randomization		X								
IP Dispensed[r]		X	X	X	X	X	X	X		
IP Returned/Accountability & Compliance Assessed			X	X	X	X	X	X	X	
Stool Assessment[t]		X	X			X		X	X	X
Adverse Events[t]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X

ECG=electrocardiogram; EOS=end of study; HOMA=Homeostasis Model Assessment; IA=interim analysis; IP=investigational product; IRT=Interactive Response Technology; MRI=magnetic resonance imaging; PRO=patient-reported outcome.

[a] Visit Windows (calculated from Visit 2): Bi-weekly (Visits 3-4): +/- 3 days; Monthly (Visits 5-6): +/-5 days; Tri-monthly (Visits 7-9/DC): +/- 7 days.

[b] Subjects will be reminded not to eat prior to their scheduled visit. Additionally, during the double-blind treatment period, they should not take their study drug prior to the visit. They should bring their study drug with them to the visit to take 30 minutes prior to their first meal of the day containing approximately 10-20 grams of fat. Also see Section 6.2.3

[c] Height to be measured at Screening only.

[d] BMI to be calculated programmatically by the Sponsor or designee for the following visits: Screening (Visit 1), Baseline (Visit 2), Visits 7 and 9/DC.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9 EOS	10 In-Clinic
Week	-8 to 0	0	2	4	8	12	24	36	48	52
Study Day	-56 to -1	0	14	28	56	84	168	252	336	364

[e] Vital signs to include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate.

[f] Biopsy performed within 6 months of Screening can be used. All biopsies will be centrally read by a hepatic histopathologist.

[g] MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.

[h] MRI at Visit 7 (Week 24) and at Visit 9 (Week 48) for the Interim Analysis Set only.

[i] All blood tests are fasting blood tests.

[j] HOMA2-IR and HOMA2-%B: will be calculated programmatically by the Sponsor or designee for the following visits: Screening (Visit 1), Baseline (Visit 2), Visits 7, 9/DC, and 10.

[k] Urinalysis to include oxalate testing.

[l] For all females of child-bearing potential (FOCP). Positive on-site urine dipstick results must have serum β-HCG testing performed by central lab. Additional testing can be performed at the investigator's discretion.

[m] Lipid Panel includes fasting total cholesterol, HDL-C, LDL-C, and triglycerides.

[n] Full coagulation panel will be done at Screening and Baseline, but only INR and PT are required at remaining time points.

[o] Serum Liver-Related Blood Tests include ALT, AST, ALP, GGT, and total bilirubin.

[p] Hepatitis B/C testing includes HBcAb, HBsAg, HBVDNA and HCVAb, HCVRNA, respectively.

[q] Thyroid testing includes thyroid stimulating hormone (TSH) and triiodothyronine (T3).

[r] Investigational product may be dispensed at an unscheduled visit outside of this schedule as needed to replace lost or damaged product.

[s] Subjects will be queried about the number of stool evacuations during the 24- hour period before the clinical research center (CRC) visit and asked to describe the consistency of the softest stool during that 24- hour period using the Bristol Stool Chart.

[t] Adverse events will be collected beginning from the signing of informed consent. All AEs must be followed to closure (the subject's health has returned to his/her Baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

1. BACKGROUND INFORMATION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide (Vernon et al. 2011), and is estimated to occur in 30-40% of adults in the United States and up to 30% of European adults. These numbers approach 95% in those with morbid obesity (Mathus-Vliegen et al. 2012). NAFLD ranges from simple steatosis, which is typically nonprogressive, to nonalcoholic steatohepatitis (NASH), which has a 20% likelihood of progression to advanced disease including fibrosis, cirrhosis and its complications including liver failure and the need for a liver transplant (Angulo 2002). Hepatocellular carcinoma is also a complication of NASH that may occur with or without the presence of cirrhosis (Torres et al. 2012). NAFLD is typically associated with type 2 diabetes mellitus (T2DM) (Adibi et al. 2007; Loomba et al. 2012), central or visceral obesity (Souza et al. 2012), dyslipidemia (Assy et al. 2000; N.C.E.P. 2002), and hypertension (Donati et al. 2004). Together these conditions comprise the metabolic syndrome, and NASH is considered to be the hepatic component of this syndrome (Hamaguchi et al. 2005, Neuschwander-Tetri 2005, Marchesini et al. 2005). Notably, NAFLD is a clinical condition occurring in individuals who do not drink excessive alcohol (>20 grams/day), yet have hepatic histology which is indistinguishable from that seen with alcoholic excess. The pathophysiology of NASH is likely multifactorial and may include combinations of metabolic, genetic, environmental, and gut microbial factors.

Most individuals with NASH are asymptomatic or have nonspecific symptoms such as fatigue. They typically first come to medical attention incidentally following routine blood testing or on imaging studies performed routinely or during the evaluation of an unrelated condition. While ultrasound and magnetic resonance imaging (MRI) can detect the presence of steatosis (Reeder et al. 2011), a liver biopsy is required to diagnose NASH and the extent of liver fibrosis.

1.1 Indication and Current Treatment Options

There are currently no drugs approved for the treatment of NASH and it is estimated that there are between 6-16 million people in the United States with NASH, of which 600,000 have severe disease (Williams et al. 2011 and Torres and Harrison 2008), with similar percentages reported throughout most areas of the world (World Gastroenterology Organisation 2012). Treatment of associated metabolic comorbidities, weight reduction, and incorporation of an exercise routine remain the cornerstone of management. However, lifestyle changes are seldom successful. Thus, NASH represents a disease with an unmet medical need that is growing at an epidemic rate, and that if untreated, carries a risk of significant morbidity and mortality.

1.2 Product Background and Clinical Information

Volixibat potassium (SHP626; formerly LUM002), hereafter referred to as volixibat, is a highly selective inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that is being evaluated for the treatment of NASH.

Bile acids (BA) promote bile flow, activation of digestive enzymes, and micellization of fats and fat-soluble vitamins, thereby permitting their intestinal absorption. BAs serve as signaling molecules acting via receptors, such as farnesoid X receptor (FXR) and transmembrane G

protein-coupled receptor (TGR5), in the intestine, liver and other tissues which play an important role in regulating insulin homeostasis. (Halilbasic et al. 2013). Lipid peroxidation and oxidant stress have been proposed as one link between the accumulation of fat and subsequent injury (Day and James 1998). All of these metabolic actions have important effects to prevent the ongoing liver damage in NASH.

High fat diet (HFD) fed mice treated with an ASBT inhibitor (ASBTi) (SC-435 a surrogate SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved insulin resistance (IR), and decreased NAS, predominantly steatosis. In addition the BA pool reflected increases in BA that were agonists to FXR and decreases in levels that were antagonist to the FXR (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015). Miethke and colleagues (2016) found that pharmacological inhibition of the ileal, ASBT (SC-435 the surrogate molecule of volixibat), blocked progression of sclerosing cholangitis in mdr2–/– mice. Beneficial effects in liver histology in these mice included a reduction in the severity of hepatic fibrosis, a decrease which correlated with a reduction of hepatic profibrogenic gene expression. While very encouraging, whether or not the findings in this mouse sclerosing cholangitis model can translate to histologic improvements in patients with NASH is unknown.

Volixibat, as a potent inhibitor of ASBT, increases BA excretion and facilitates signaling in the intestine that regulates serum and hepatic BA concentrations, glucose metabolism, serum cholesterol and fatty acid metabolism in the liver. The combined events resulting from inhibiting BA reuptake are hypothesized to have a positive metabolic, anti-inflammatory, anti-steatotic, and potentially antifibrotic effect that will lead to a therapeutic benefit for patients with NASH.

One of the factors contributing to the pathogenesis of NASH is abnormal cholesterol metabolism and the accumulation of free cholesterol in the liver. The free cholesterol is directly toxic to hepatocytes, which leads to inflammation and fibrosis (Musso et al. 2013). One treatment approach is to remove the cholesterol from the liver to decrease and possibly reverse the damage to the hepatocytes. This is one of the mechanisms by which SHP626 will treat NASH.

Due to the mechanism of action (MOA), volixibat is under development for the treatment of NASH and may also be able to improve the metabolic syndrome that is associated with NASH. Volixibat inhibits ASBT therefore BAs are excreted in the feces and this loss forces the liver to synthesize new BA which utilizes cholesterol in the liver and serum. Volixibat also has the potential to reduce IR, which is considered to be the most common underlying risk factor for the development of NASH (Pagano et al. 2002; Sanyal et al. 2001).

Volixibat was initially being evaluated for its use as an intervention for dyslipidemia. As a result of initial observations in animals that serum low-density lipoprotein cholesterol (LDL-C) and total liver cholesterol content could be decreased after administration of volixibat, additional work was undertaken to support the safety and tolerability of the drug in healthy volunteers. Clinical and non-clinical studies have demonstrated very low systemic exposure across species. Oral administration of volixibat at doses up to 300 mg once daily and 50 mg administered daily for 14 days to healthy male subjects was generally safe. A Phase 1 study revealed that volixibat is tolerated at the 10 mg dose for 28 days and that there were trends towards increasing high-

density lipoprotein cholesterol (HDL-C), decreasing LDL-C, and decreasing fasting glucose in patients with T2DM (LUM002-101 trial).

A healthy volunteer study investigating 12 days of varying levels of repeat doses of volixibat has provided key pharmacodynamic information for dose selection as measured by the effect of volixibat on excreted fecal bile acid (FBA) levels (SHP626-101). In addition, the study investigated safety and tolerability over the dose range that was considered for this Phase 2 study.

Data from Phase 1 studies support the position that volixibat is basically a non-absorbed drug that works locally in the GI tract and results in virtually no systemic exposure. Thus pharmacokinetic sampling will not be done in this study.

The current proof of concept (POC) Phase 2 trial will evaluate the safety, tolerability, and efficacy of three doses of volixibat (5, 10, and 20 mg) in adult subjects with NASH. Due to the MOA of volixibat, efficacy will be assessed at an interim analysis (IA) by a change of steatosis from Baseline to Week 24 compared to placebo (PBO). While the gold standard for quantification of steatosis has historically been an invasive liver biopsy, quantitative magnetic resonance (MR) imaging–based biomarkers for liver fat have evolved rapidly over the last decade, and are increasingly being incorporated into NASH clinical trials. Both MR spectroscopy (S) and MR imaging (I) proton density fat-fraction (PDFF) provide non-invasive means of quantifying intrahepatic lipid content (Reeder et al. 2012). Both techniques have been shown to be accurate, reproducible with a low degree of variability in interpretation, cost-effective and reliable biomarkers of quantitative hepatic fat (Roldan-Valadez et al. 2010; Urdzik et al. 2012; Raptis et al. 2012). Importantly, studies demonstrate a close correlation with steatosis grade histologically (Qayyum et al. 2005; Schwenzer et al. 2009).

In a study of 51 adult subjects with NAFLD, PDFF correlated well with the grade of histologic steatosis, as the mean fat-fraction values of 8.9%, 16.3%, and 25.0% corresponded to histologic steatosis grades 1, 2, and 3, respectively ($P < .0001$) (Permutt et al. 2012). Thus, MRI will be utilized to evaluate the degree of steatosis change from Baseline in this study during the IA. The Mozart trial was a randomized, double-blind, PBO-controlled trial of 50 patients with NASH who were randomized to 24 weeks of either ezetimibe or PBO, evaluating the reduction of liver fat by MRI-PDFF as well as by histology. Results revealed that compared to histologic non-responders, histologic responders, defined as a two-point reduction in NAFLD activity score (NAS) without worsening fibrosis, had a statistically significant reduction in net MRI-PDFF of -4.1% ± 4.9 vs. +0.6% ± 4.1 ($P < 0.036$) with a mean percent change of -29.3% ± 33.0 vs. +2.0% ± 24.0 ($P < 0.004$), respectively (Loomba et al. 2015). Thus, in the current trial during the IA, an active dose will be considered to be efficacious if a ≥ 5% steatosis reduction compared to PBO is seen after 24 weeks of therapy.

Intrahepatic lipid content of less than 1% is considered to be within the normal range (Springer et al. 2015), however, from a study of 2349 people in a general population undergoing MRS, it was concluded that a PDFF value of 5.56% represented the upper limit of the normal range, as determined from the 95th percentile of PDFF in 345 individuals who were not at increased risk for hepatic steatosis (Szczepaniak et al. 2005). Thus, in the current trial, similar to other NASH

trials utilizing MR for evaluation, an MRI ≥5% steatosis will be used as an inclusion criterion (Loomba et al. 2015).

1.3 Benefits and Risks

By virtue of volixibat's ability to inhibit ASBT bile acid reabsorption, there is an increase in BA excretion and signaling in the intestine that results in improvements in glucose metabolism and changes in cholesterol and fatty acid synthesis in the liver. Recently, HFD fed mice treated with an ASBTi (SC-435 a surrogate of SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved IR, and decreased NAS (predominantly steatosis). In addition, these HFD-fed mice did not gain weight when treated with SC-435, in spite of consuming increased calories. Finally, the BA pool in these mice changed to predominantly FXR agonist (Karpen et al. oral presentation AASLD 2015; Rao et al. 2015).

These metabolic actions and preclinical results may prove to be clinically relevant to subjects with NASH.

NASH has recently received considerable attention as awareness of the problem of liver damage and prevalence of the disorder has increased, paralleling the obesity epidemic. Consequences of liver damage are detrimental and can lead to liver failure, hepatocellular carcinoma, and the need for liver transplantation. There is no currently approved medical therapy for NASH. The large unmet medical need and the increased medical resource burden have led to the search for potential therapies to treat NASH.

Nonclinical testing established that the no observed adverse effect level (NOAEL) for volixibat in rats and dogs following 13 weeks of once-daily administration were 1000 and 500 mg/kg/day, respectively. Similarly, testing confirmed that the NOAEL for volixibat in a 6-month study in rats and a 9-month study in dogs were 1000 and 500 mg/kg/day, respectively. In both cases, these were the highest doses tested. Genotoxicity testing has yielded negative findings.

Volixibat is minimally absorbed. The pharmacokinetic profiles performed in clinical studies completed to date repeatedly suggest negligible systemic exposure. Furthermore, there has been no observation of clinically relevant changes in fat absorption parameters such as those related to fat-soluble vitamins.

The most frequent TEAEs in the Phase 1 studies were GI and were considered mechanism-based due to elevated BA concentrations in the colon. The percentage of subjects reporting at least 1 TEAE in the GI disorders SOC generally increased with an increasing volixibat dose level (Part 1 Study TDU10632 and Study LUM002-101). Most TEAEs were mild in intensity, and none were assessed as severe.

In the multiple dose studies, the most commonly reported TEAEs in subjects (both healthy and with T2DM) who received volixibat for the longest duration of 28 days in Study LUM002-101 included diarrhea and abdominal pain. The most commonly reported TEAEs in subjects receiving 50 mg volixibat for 14 days (part 3 Study TDR10633) were diarrhea and GI pain.

Overall, there were 2 SAEs (ALT increased and retinal detachment), both of which led to the discontinuation of volixibat. In part 3 of the initial Phase 1 study (TDU10633), 1 subject dosed with 50 mg volixibat for 13 days was withdrawn from the study due to a mild TEAE (which became a SAE due to prolonged hospitalization) of ALT increased that was considered related to volixibat . The subject's ALT level returned to normal after discontinuation of volixibat. A second subject dosed with 10 mg volixibat for 12 days in Study LUM002-101 reported a moderate SAE of ablation of the retina with a bleed in the vitreous body of the right eye that was considered not related to volixibat.

Overall, 3 subjects, all dosed with 5 mg volixibat in Study LUM002-101, discontinued volixibat due to non-serious TEAEs: 1 due to a related TEAE of mild hemorrhagic diarrhea, 1 due to an unrelated TEAE of moderate Epstein-Barr virus infection, and 1 due to mild related TEAEs of diarrhea and anal erosion.

Overall, the observed AEs attributable to volixibat have been self-limited as would be expected given the local MOA of ASBT inhibition in the terminal ileum. Generally, among subjects who experienced GI TEAEs, the events have been mild and diminished over the course of treatment. Please refer to the investigator's brochure for additional information.

Volixibat is a novel drug candidate, demonstrating limited systemic exposure across species with the potential to affect important metabolic pathways associated with NASH. The overall safety, tolerability, and preliminary activity of volixibat in available clinical trials suggest that further investigation is warranted and that there is a positive benefit to risk profile.

Always refer to the latest version of the Volixibat Potassium (SHP626) Investigator's Brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of volixibat.

See Appendix 1 for protocol history, including all amendments.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Currently there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its MOA and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

2.2 Study Objectives

2.2.1 Primary Objective

To evaluate the effect of volixibat compared to PBO on liver histology

2.2.2 Secondary Objectives

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual NAS components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

2.2.3 Exploratory Objectives

- To explore the effect of volixibat compared to PBO on liver histology (measured by individual SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F))
- To explore the effect of volixibat compared to PBO on anthropometric measures (body weight, body mass index (BMI), waist circumference and waist-hip ratio)
- To explore the effect of volixibat compared to PBO on homeostasis model assessment 2-IR (HOMA2-IR) and HOMA2-beta cell function (HOMA2-%B) in subjects with T2DM
- To explore the effect of volixibat compared to PBO on BA synthesis (7-alpha-hydroxy-4-cholesten-3-one [C4])

- To explore the effect of volixibat on patient-reported health-related quality of life (HRQoL) and overall health status.

3. STUDY DESIGN

3.1 Study Design and Flow Chart

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio such that a target of 266 subjects is achieved (221x1.2 is approximately 266). The 221 subjects include 201 subjects for the three arms analyzed at 48 weeks plus an additional 20 subjects to account for one dose dropped at the IA). Attempt will be made to perform the IA before any subject has had their 48-week post-treatment liver biopsy, although this will be dependent upon the rate of enrollment and dropout.

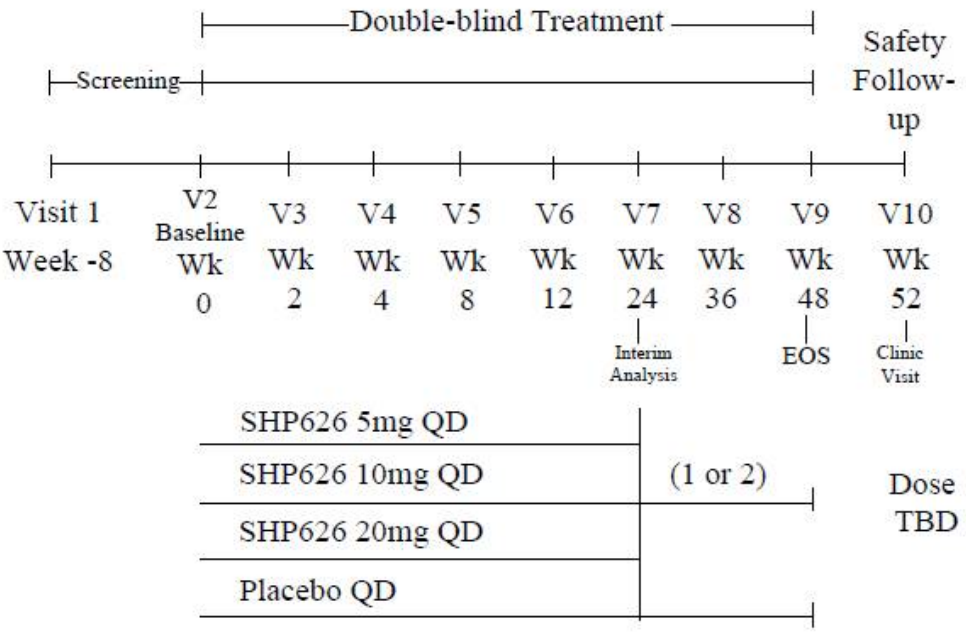
There will be up to 3 periods (Screening, Treatment and Follow-up) and an IA. The duration of the Treatment period will be 48 weeks, with an IA at Week 24. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

The IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. Study enrollment will be paused after 92 subjects have been randomized. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO, on reduction of steatosis as measured by high fat fraction (HFF) by MRI and ALT.

Once decisions as to dose elimination are made, study enrollment will be reopened and additional sites may be added. Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining dose groups or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of investigational product (IP).

The study will be conducted over 3 periods: Screening (8 weeks), Treatment (48 weeks), and Follow-up (4 weeks), with an IA at 24 weeks as outlined in Figure 1.

Figure 1: Study Design Flow Chart



3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 364 days. The last visit is an in-clinic safety follow-up visit. The study will be completed in approximately 3 years.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at approximately 60 to 80 clinical sites in the USA, Canada, and EU.

4. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first Screening visit.
4. Males and females of child-bearing potential who are sexually active, or are not currently sexually active during the study, but become sexually active during the period of the study and 30 days following the last dose of the IP, must agree to use acceptable contraception as described in Section 4.4, Reproductive Potential.

   Males and females of child-bearing potential who are not currently sexually active must agree to use acceptable contraception, if they become sexually active during the period of the study and 30 days following the last dose of the IP.
5. Presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis (F1-F3) from a centrally read liver biopsy performed either during the Screening period or within 6 months prior to the first visit with a NAS of ≥ 4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met.

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV (defined as HCVAb positive without a current or prior history of a detectable HCVRNA) may be eligible alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis,

Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.

3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the investigational product (IP) (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥ 5% after qualifying liver biopsy performed.
5. Contraindications to MRI (eg, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study.
7. Treatment with Vitamin E, thioglitazones, or glucagon-like peptide-1 receptors agonists (GLP1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation (refer to Section 5.2.1, Permitted Treatment).
8. Uncontrolled diabetes defined as HbA1c of ≥ 9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of Screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to Screening. Also refer to Section 5.2.2, Prohibited Treatment.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to liver biopsy, or initiated any time after liver biopsy performed, including the use of total parenteral nutrition (TPN). Also refer to Section 5.2.2, Prohibited Treatment.
11. Serum AST > 5 times upper limit of normal (ULN) at Screening.
12. Serum ALT > 5 times ULN at Screening.
13. Elevated serum creatinine ≥ 2.0 mg/dL.
14. International normalized ratio (INR) >1.3
15. TB ≥ 2.0 x ULN at Screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count < 130×10^9/L
17. Medical history of impaired hemostasis or use of anticoagulant medication (low-dose, ie 81 mg, aspirin (ASA) will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.

20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year or at any time during the study based on investigator's discretion. Refer to Section 7.2.3.8, Drug and Alcohol Screening. Weekly alcohol intake greater than 21 grams/day for males and 14 grams/day for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
25. Known positivity for human immunodeficiency virus (HIV) infection.
26. Cancer within 5 years of Screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
27. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
28. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
29. Subject has failed Screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
30. Subjects who are employees at the investigational site.

4.3 Restrictions

Subjects must adhere to the following restrictions for the duration of the study:

- Subjects must remain compliant with inclusion/exclusion criteria.
- Subjects should not become pregnant, father a child, or nurse/breastfeed a baby.
- Subjects should be encouraged to adhere to the same exercise routine and a healthy diet throughout the study.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of child-bearing potential should be using an acceptable form of contraception. Females of child-bearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of the IP. If hormonal contraceptives are used they should be administered according to the package insert. Females of child-bearing potential who are not currently sexually active must agree to use acceptable contraception, as defined below, if they become sexually active during the period of the study and 30 days following the last dose of the IP.

Female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and age ≥ 51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of child-bearing potential with a negative urine and/or serum β-human chorionic gonadotropin (β-HCG) pregnancy test each study visit. Females of child-bearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception.

Acceptable methods of contraception are:

- Intrauterine devices(IUDs)
- Barrier methods (eg, condoms or diaphragms with spermicidal gel or foam)
- Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring), stabilized for at least 30 days prior to the Screening visit (Visit 1), plus condoms. Note: if subject becomes sexually active during the study, they should use one of the other acceptable methods noted above in addition to the hormonal contraceptive until it has been stabilized for 30 days.
- Male sterilization
- True abstinence (ie, periodic abstinence and withdrawal are not acceptable)

4.4.2 Male Contraception

Contraception is required for all sexually-active male subjects and their partners. All male subjects (including those who are sterile) agree not to donate sperm, and to use 1 of the following approved methods of contraception from the Baseline visit on Day 0 until 30 days following study discharge:

- Male condom with spermicide
- Sterile sexual partner
- Intrauterine device with spermicide (use by female sexual partner)
- Female condom with spermicide (use by female sexual partner)
- Contraceptive sponge with spermicide (use by female sexual partner)
- Intravaginal system (eg, vaginal ring with spermicide, a diaphragm with spermicide, or a cervical cap with spermicide) (use by female sexual partner)
- Oral, implantable, transdermal, or injectable hormonal contraceptive (use by female sexual partner).

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from the IP with the CRO Medical Monitor when possible.

If the IP is discontinued, regardless of the reason, the evaluations listed for both Visit 9/end of study (EOS) and Visit 10/Follow-up, are to be performed as completely as possible; however, the EOS liver biopsy will not be required for patients discontinuing prior to Week 44.

Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination, date of stopping the IP, and the total amount of the IP taken must be recorded in the electronic case report form (eCRF) and source documents.

Subjects who discontinue will not be replaced.

4.5.1 Subject Withdrawal Criteria

Medically important events that in the opinion of the investigator, medical monitor or sponsor would compromise the subject's ability to safely continue in the study may result in withdrawal of the subject from the study.

4.5.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the eCRF. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event (AE)
- Protocol violation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Screen failure
- Noncompliance with study drug
- Physician decision
- Pregnancy
- Progressive disease
- Study terminated by sponsor
- Other - If "Other" is selected, the investigator must specify on the eCRF

4.5.3 Subjects Lost to Follow-up Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact clinic visit. At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

4.5.4 Safety-related Stopping Rules

Refer to Appendix 4 and Appendix 5 for criteria to assess severity of liver-related AEs and stopping rules for liver-related blood tests, respectively.

An urgent safety review will be conducted within 7 days by the sponsor if one or more of the following criteria are met:

- Death that is considered related to the study drug
- Two SAEs of similar type (defined as same or similar Medical Dictionary for Regulatory Activities (MedDRA) higher level group code), and considered related to the study drug.

The urgent review will be performed by a sponsor safety review group, which will include the study Pharmacovigilance and Risk Management (PVRM) Medical Lead and the PVRM therapeutic area (TA) Head. The PVRM TA Head, not the PVRM Medical Lead involved in the study, may be unblinded as part of this urgent safety review, if required. Following the sponsor's review of safety data, one of the following actions will be taken with respect to study status:

- Continue study with protocol unchanged
- Continue study with modifications to the protocol
- Terminate study

Subject safety will be monitored on a continuous basis during this study until the last subject completes his or her last scheduled study visit/assessment.

5. PRIOR AND CONCOMITANT TREATMENT

All non-study treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to signing informed consent at the Screening visit (Visit 1) and through the final study contact (including protocol-defined follow-up period) must be recorded on the appropriate eCRF page.

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, and behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate), received within 30 days of the date of first dose of the IP. Prior treatment information must be recorded on the appropriate eCRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of the IP and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate eCRF page.

5.2.1 Permitted Treatment

Medications and supplements including but not limited to vitamin E, betaine, s-adenosyl-l-methionine, ursodeoxycholic acid, milk thistle, gemfibrozil, anti-TNF therapies, probiotics biguanides (metformin), thiazolidinediones (TZDs) and GLP1 RAs that have been used to treat NAFLD/NASH are allowed during the course of the study if the subject has been on a stable dosing regimen (ie, same dose and frequency in the previous 6 months prior to the qualifying liver biopsy and not initiated after qualifying liver biopsy) and will continue this dosing regimen throughout study participation. The investigator must contact the CRO medical monitor then the Shire GCDL to discuss any changes to concomitant medications that may impact the study.

5.2.2 Prohibited Treatment

The following list of prohibited drugs cannot have been taken for more than 2 weeks within the year prior to randomization and are excluded while on study.

- Systemic Glucocorticoids
- Tamoxifen
- Amiodarone
- Methotrexate
- Alcohol (see Section 4.2)
- Griseofulvin
- Total parenteral nutrition
- Valproate
- Nucleoside Analogues
- Tetracycline (high dose)
- Estrogens at doses greater than used for hormone replacement
- Anabolic steroids
- Bile acid sequestrants such as

cholestyramine or colestipol

- Any other known hepatotoxins including over-the counter therapies and herbal therapies such as germander, chaparral and ma-huang.

This is not a comprehensive list. Treatments not listed above are generally considered allowable, unless considered a potential hepatotoxin. Antidiarrheals will be allowed at the discretion of the investigator, with the exception of BA sequestrants.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is volixibat potassium (formerly LUM002, formerly SHP626, hereafter referred to as volixibat) which will be provided in 5, 10 and 20 mg capsule form. Additional information is provided in the current Volixibat Potassium (SHP626) Investigator's Brochure.

The reference/comparator product is an identical PBO which will be provided in capsule form.

6.1.1 Blinding the Treatment Assignment

The IP will be supplied as double-blind blister packs. The actual double-blind treatment given to individual subjects is determined by a randomization schedule which will be automatically assigned by the interactive response technology (IRT). Placebo capsules, which exactly match the IP, will be used in the blister packs to provide the same number and size capsules for each of the doses within the treatment groups.

6.2 Administration of Investigational Product(s)

All IP and supplies will be provided by Shire or its designee**.** At each visit, subjects will be supplied with enough IP to last until the subsequent visit. Lost or damaged IP will be replaced as needed. Volixibat will be supplied to the clinical research center (CRC) as powder in capsule. Volixibat will be supplied in identical capsules in strengths of 5, 10, and 20 mg (with matched PBO).

6.2.1 Interactive Response Technology for Investigational Product Management

IRT will be used for the following investigational tasks:

- Randomization
- Supply management
- Inventory management and supply ordering
- Expiration tracking
- Returns
- Emergency unblinding

6.2.2 Allocation of Subjects to Treatment

This is a double-blind, PBO-controlled study. The actual treatment given to individual subjects is determined by a randomization schedule.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation.

The randomization number represents a unique number corresponding to the IP allocated to the subject, once eligibility has been determined.

Individual subject treatment is automatically assigned by the IRT.

Once a randomization number/unique identifier has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. If a randomization number/unique identifier is allocated incorrectly, the clinical research associate (CRA)/study monitor must be notified as soon as the error is discovered.

IP packaging identification numbers, separate from randomization numbers/unique identifiers, may also be assigned to subjects for specific treatment assignment as dictated by the study. In these cases, the same IP packing identification number may not be assigned to more than 1 subject.

Subjects will be equally allocated to all volixibat doses and PBO if their randomization precedes the 24 week IA. If a subject's randomization occurs after the 24 week IA, subjects will be equally allocated to all remaining volixibat doses (determined based on the results of the IA) and PBO. The randomization will be stratified by treatment arm, baseline T2DM, and NAS ≥ 6 or NAS = {4, 5}.

6.2.3 Dosing

All doses of volixibat or matching PBO will be administered orally as a capsule in a double-blinded fashion. The first dose of IP for each subject will be administered in the clinic. The dose will be administered with 240 mL of water and should be given 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat. All assessments should be completed at least 30 minutes prior to administration of the IP. The subject should make all attempts to consistently take the IP around the same time each day. If a dose is missed at the normally scheduled time, the subject can make up the dose that day; however, they should not take two doses in any 24-hour period.

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be broken during the study except in emergency situations where the identification of the IP is required for further treatment of the subject. The investigator should contact the CRO medical monitor and the Shire GCDL at the same time and as soon as possible after the investigator has been unblinded.

In the event that the treatment assignment is broken, the date and the signature of the person who broke the code are to be recorded in the source documents and the IRT, and the reason for

breaking the code will be recorded in the source documents and the clinical database. Upon breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code-breaks that occur must be reported to the CRO medical monitor. Code-break information is held by the pharmacist/designated person at the site and by the CRO medical monitor for the study or designee.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled with a minimum of the protocol number, medication identification number, dosage form (including product name and quantity in pack), directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements 'For clinical trial use only', and/or 'CAUTION: New Drug - Limited by Federal (or USA) Law to Investigational Use', 'Keep out of reach of children', and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Additional labels may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The sponsor or designee will provide the IP for this study. The IP is packaged in the following labeled containers:

- Volixibat 5 mg capsules
- Volixibat 10 mg capsules
- Volixibat 20 mg capsules
- Volixibat PBO capsules

6.3.3 Storage

The investigator has overall responsibility for ensuring that the IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented.

IPs are distributed by the pharmacy or nominated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP labels as they are distributed.

All IP must be stored at the clinic at 20 - 25°C (68 - 77°F); excursions are allowed between 15 - 30°C (59 - 86°F).

IPs must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the IP is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion below 15°C (59°F) or above 30°C (86°F); these excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room or a change in storage location.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing the IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed IP will be documented on the eCRFs and/or other IP record. The investigator is responsible to ensure the retrieval of all study supplies from subjects.

No IP stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records. The site must ensure that the accountability and destruction records are complete, accurate, and ready for verification at each monitoring visit.

The process for return and destruction of IP must be determined and documented during the study start-up phase.

With the written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject returned IP, and empty/used IP packaging may be destroyed at the site or a local facility on an ongoing basis throughout the study and at the end of the study following verification of accountability by the CRA/study monitor. In this case, destruction records identifying what was destroyed, when, how, and by whom, must be obtained with copies provided to the sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Alternatively, in the absence of written agreement by the sponsor of the site's IP destruction procedures, all unused stock, subject-returned IP, and empty/used IP packaging may be required to be sent to a nominated contractor on behalf of the sponsor for IP destruction on an ongoing basis throughout the study and at the end of the study. IP being returned to the sponsor's designated contractors also must be counted and verified by clinical site personnel and the CRA/study monitor. For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, IRT) do not require a shipment form. Returned IP must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any IP prior to shipment. Shipment of all returned IP must comply with local, state, and national laws.

6.5 Subject Compliance

Subjects must be instructed to bring their unused IP and empty/used IP packaging to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, blister pack) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details on the drug accountability form.

7. STUDY PROCEDURES

7.1 Study Schedule

This study will consist of a Screening period of up to 56 days (the screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor), a 48-week Treatment period, and a Follow-up visit 4 weeks after treatment ends. A detailed display of all study procedures is provided in Table 1: Schedule of Assessments.

Patients will be assessed according to the following schedule:

- Screening – Visit 1 (Weeks -8 to 0 [Day -56 to Day -1])
- Baseline – Visit 2 (Week 0 [Day 0])
- Treatment and Assessments – Visits 3 through 9 (Week 2 through Week 48)
- Follow-up – Visit 10 (Week 52, 4 weeks after completion of dosing)

7.1.1 Screening (Visit 1)

Screening procedures must be completed within 56 days prior to randomization for the first dose of the IP. The screening period may be minimally extended under special circumstances only with the explicit approval of the medical monitor. At the Screening visit, considered Visit 1 (Week -8 to 0, Day -56 to -1), all screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from the subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

The following Screening procedures should be assessed at the beginning of the visit window, preferably between Day -56 and Day -49: inclusion/exclusion criteria, collection of subject information (demographics, medical and medication history AEs, and concomitant medications), a physical examination including vital signs, height, weight, and waist/hip measurements; collection of blood and urine samples for screening and safety assessments, performance of an electrocardiogram (ECG), and scheduling of MRI and liver biopsy. MRI and liver biopsy (if one has not been completed in the previous 6 months) should be performed with sufficient time to ensure results are received prior to the Baseline visit.

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered the IP. Subjects cannot be rescreened once they have been designated as a screen failure.

7.1.2 Baseline (Visit 2)

Following the Screening visit, subjects will return to the clinic within 56 days for Visit 2, (considered Week 0, Day 0), for Baseline assessments including review of the inclusion/exclusion criteria, adverse events, and concomitant medications; physical examination with vital signs, weight, and waist/hip measurements; ECG; collection of blood and urine samples; and completion of the patient-reported outcome (PRO) EuroQol-5 Dimension-5 Level Questionnaire (EQ-5D-5L) survey. After completion of these assessments, subjects are randomized using Interactive Response Technology (IRT) to 1 of 4 treatment arms receiving volixibat 5 mg, 10 mg, or 20 mg, or PBO. The initial supply of study drug is dispensed to ensure adequate daily dosing until the next scheduled study visit.

7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)

During the 48 weeks of double-blind treatment, 7 clinic visits are scheduled to occur as follows:

- Visit 3 - Week 2, Day 14 (+/- 3 days)
- Visit 4 - Week 4, Day 28 (+/- 3 days)
- Visit 5 - Week 8, Day 56 (+/- 5 days)
- Visit 6 - Week 12, Day 84 (+/- 5 days)
- Visit 7 - Week 24, Day 168 (+/- 7 days) – Interim Analysis
- Visit 8 - Week 36, Day 252 (+/- 7 days)
- Visit 9 - Week 48, Day 336 (+/- 7 days) – End of Study

Subjects are reminded not to eat and not to take their study drug on the day of scheduled study visits prior to completion of assessments. They should bring their study drug with them to the visit. All assessments should be completed at least 30 minutes prior to administration of the IP. IP should be taken 30 minutes prior to the first meal of the day containing approximately 10-20 grams of fat.

The Schedule of Assessments provided in Table 1 details the procedures to be completed at each visit. All Treatment visits (3 through 9) will include weight and waist/hip measurements, assessment of vital signs, and blood sampling for completion of biochemistry, hematology, serum glucose, lipid panel, HbA1c, and serum liver-related blood tests. At all visits, adverse events, and concomitant medications will be collected for all subjects, and female subjects of childbearing potential will have a urine pregnancy test. Subjects will return containers of unused study drug for assessment of accountability and compliance which will be documented in the IRT. New supplies of study drug will be dispensed at Visits 3 through 8.

Additional assessments will also occur less frequently during the Treatment Period for coagulation panel, vitamin D, insulin, thyroid testing, urinalysis, ECG, physical examinations, and completion of the EQ-5D-5L.

An MRI will be repeated at Visit 7 for the 24-week IA and at Visit 9 (Week 48) for the subjects in the IA set only. A final liver biopsy will be performed at Visit 9 (Week 48) for all subjects unless a subject discontinues prior to Week 44, in which case the EOS liver biopsy is not required.

7.1.4 Follow-up (Visit 10)

The follow-up period for this protocol is 4 weeks after the last dose of study drug with a final Follow-up visit scheduled for Week 52. Procedures to be completed at this final visit include physical examination, weight, waist/hip measurements, vital signs, samples for biochemistry and hematology, serum glucose, lipid panel, HbA1c, serum liver-related blood tests, insulin, thyroid testing, and urine pregnancy test (for women of childbearing potential). Adverse events and concomitant medications will be recorded. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.5 Additional Care of Subjects after the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

All assessments and procedures are to be performed by the Investigator or a qualified designee who has been trained in the protocol. Assessments are to be performed according to the schedules shown in Table 1. If a subject terminates the study early, the CRC will make reasonable effort to perform the EOS and safety follow-up assessments and procedures for the subject's safety and well-being.

7.2.1 Demographic and Other Baseline Characteristics

Demographic details will be obtained at screening and recorded on the eCRF. Data collected will include age, gender, ethnicity, height, and weight.

7.2.2 Efficacy

7.2.2.1 Liver Biopsy

Liver biopsies will provide histologic data for confirmation of the diagnosis of NASH, assessment and grading of NASH activity, and scoring of steatosis, lobular inflammation, ballooning, as well as fibrosis and additional features (see Appendix 2 and Laboratory Manual for additional information).

7.2.2.2 MRI

The presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist will be performed either during the Screening period or within 6 months prior to the first visit. Steatosis is assessed by MRI hepatic fat fraction. The Week 24 and Week 48 MRIs will be conducted for subjects in the IA set only.

7.2.3 Safety

7.2.3.1 Medical History and Medications

Medical history including important medical events and concomitant medication and illnesses will be obtained at Screening and will be recorded on the eCRF. Any existing medical condition present prior to the time of randomization should be reported as medical history

7.2.3.2 Physical Examination

A complete physical examination will be performed with a thorough review of body systems at Screening, Baseline prior to randomization, and at study visits specified in Table 1. Physical examinations will include a review of the subject's general appearance, as well as evaluation of the body systems including:

- Eyes, ears, nose, throat
- Lymph nodes
- Cardiovascular
- Skin
- Abdomen
- Neurological
- Spine and extremities

Abnormalities identified at the Screening visit (Visit 1) will be documented in the subject's source documents and on the medical history eCRF. Changes after the Screening visit (Visit 1) will be captured as AEs on the AE eCRF page, if deemed clinically significant by the investigator.

Height will be measured at the Screening visit only while weight and waist and hip circumference will be recorded at all study visits. BMI will be calculated programmatically.

7.2.3.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.3.4 Vital Signs

Vital signs include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate. Blood pressure should be determined by cuff (using the same method, the same arm, and in

the same position throughout the study). Any deviations from Baseline (Visit 2) vital signs which are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

7.2.3.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the central laboratory's normal procedures. Reference ranges supplied by the central laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Clinically significant findings should be evaluated for recording as adverse events on the eCRF. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Blood urea nitrogen (BUN)
- C4
- Calcium (Ca)
- Bicarbonate (CO2)
- Chloride (Cl)
- Creatinine
- Creatine kinase
- Gamma glutamyl transferase (GGT)
- Glucose
- Lactate dehydrogenase (LDH)
- Magnesium (Mg)
- Phosphate (P)
- Potassium (K)
- Sodium (Na)
- Total and direct bilirubin
- Total cholesterol
- Protein
- Triiodothyronine (T3)
- Thyroid-stimulating hormone (TSH)
- Triglycerides
- Uric acid

Hematology

- Hemoglobin
- Hematocrit
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelets
- Red blood cell (RBC)
- White blood cell (WBC) count with differential
- Prothrombin time (PTT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Urinalysis

- Appearance (clarity and color)
- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopic examination of sediment
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen
- Oxalate

7.2.3.6 Additional Laboratory Assessments

Laboratory samples will be collected and assessed for:

- HIV testing and assessment of Hepatitis B/C including HBcAb, HBsAg, HBVDNA, and HCVAb, and HCVRNA will occur at the Screening visit only
- Lipid panel including fasting total cholesterol, HDL-C, LDL-C, and triglycerides at each scheduled study visit (Visits 1 through 10)
- HbA1c will be tested at every scheduled visit (Visits 1 through 10)
- Full coagulation panel will be done at Screening and Baseline, but only INR and PT are required at remaining time points at Visits 4, 7, and 9
- Insulin will be tested at Screening, Baseline, at Visit 4 and Visits 6 through 10
- HOMA2-IR and HOMA2-%B: will be calculated programmatically from serum glucose tested at Screening, Baseline, Visits 7, 9, and 10

- Vitamin D will be tested at Screening, Baseline, and Visits 4, 7, and 9
- Thyroid testing including TSH and T3 will be tested at Screening, Baseline, and Visits 4, 6, 7, 9, and 10
- C4 samples will be collected at Baseline and Visits 6, 7, 9, and 10

7.2.3.7 Pregnancy Test

A urine pregnancy test is performed on all females of child-bearing potential at the Screening visit (Visit 1), Baseline visit (Visit 2), at each Treatment visit (Visits 3 through 9) and at the Final visit (Visit 10), or if pregnancy is suspected, or on withdrawal of the subject from the study. A positive urine pregnancy test must be followed with a serum pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Section 8.1.7.

7.2.3.8 Drug and Alcohol Testing

A urine screen for drugs of abuse and blood test for alcohol will be performed at Screening and Baseline as described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the eCRF database.

Any positive result for drugs of abuse at the Screening or Baseline visits may exclude the subject from further participation in the study. A positive test for drugs of abuse or alcohol done at the investigator's discretion discovered at any time during the study will be grounds for study discontinuation.

7.2.3.9 Electrocardiogram

An ECG (12-lead) will be performed at the times specified in Table 1 in accordance with the clinical site's standard practice(s) and equipment supplied by the CRC. Recordings of ECGs will be read locally at the clinical site by the investigator or designee. The ECG will include assessments of heart rate and PR, RR, QRS, and QT intervals. Identification of any clinically significant findings and/or abnormalities will be recorded on the eCRF.

7.2.3.10 Health-related Quality of Life Assessments

The EuroQol (EuroQol. 2016. Available at: http://www.euroqol.org. [Accessed 17 February 2016]) EQ-5D-5L ("EQ-5D") is a widely used standardized questionnaire that assesses generic HRQoL and is also recommended for health-economic evaluations. The EQ-5D includes two components: a descriptive profile and a visual analogue scale (VAS). The descriptive profile includes five dimensions (ie, pain/discomfort, mobility, usual activities, self-care and

anxiety/depression), each with five levels (ie, no problems, slight problems, moderate problems, severe problems, extreme problems). An EQ-5D index can also be derived from the data which summarizes health status using a single value (ie, health-state utility). The psychometric properties of the EQ-5D-5L have been established and well documented (see Appendix 3).

7.2.3.11 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 124 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 124 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not the IP is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE eCRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of the IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of the IP, but the dyspepsia becomes severe and more frequent after first dose of the IP has been administered, a new AE of severe dyspepsia (with the appropriate date of onset) is recorded on the appropriate eCRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Appendix 4 provides criteria to assess the severity of liver-related adverse events.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to the IP for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the IP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the eCRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown.

8.1.4 Symptoms of the Disease Under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected progression of the disease and are part of the efficacy

data to be collected in the study; however, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pre-treatment value observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.6 Criteria for Discontinuation of Treatment

Appendix 5 provides guidelines for discontinuation of treatment based on elevated ALT, AST, TB, and associated signs and symptoms.

8.1.7 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.4.

Any report of pregnancy for any female study participant (or the female partner of a male participant) must be reported within 24 hours to the Shire Global Pharmacovigilance and Risk Management Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO medical monitor and the Shire clinical physician using the details specified in the emergency contact information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-HCG test or ultrasound result will determine the pregnancy onset date.

8.1.8 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of the IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- Misuse – Intentional use of the IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol)

- Overdose – Intentional or unintentional intake of a dose of an IP exceeding a pre-specified total daily dose of the product.

- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the Volixibat Potassium (SHP626) Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Pharmacovigilance and Risk Management Department and the Shire GCDL within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.8) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Pharmacovigilance and Risk Management Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the Shire GCDL using the details specified in the emergency contact information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence (whether considered to be related to the IP or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect

- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. 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.

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.4, and must be reported to the Shire Global Pharmacovigilance and Risk Management Department and the Shire GCDL within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Pharmacovigilance and Risk Management Department within 24 hours of the first awareness of the event.

8.2.4 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.5 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received the IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

8.2.6 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The CRO is responsible for notifying the relevant regulatory authorities as appropriate: USA central IRBs/EU central ECs of related, unexpected SAEs.

In addition the CRO is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP626-201 program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the eCRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Data that may potentially unblind the treatment assignment (ie, IP serum concentrations, antibiotics to investigation product, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

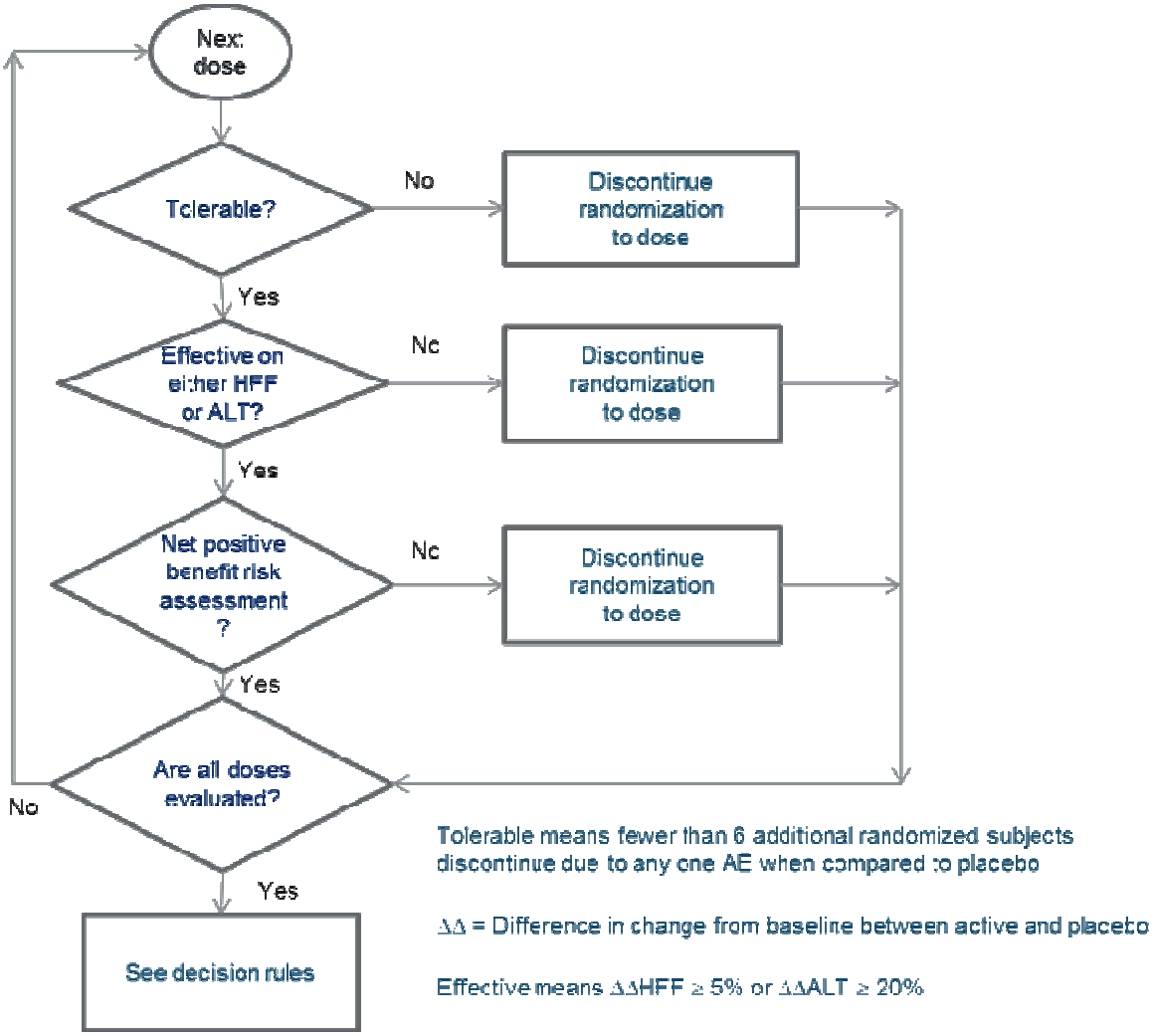
All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513).

9.5 Planned Interim Analysis

Study enrollment will be paused after 92 subjects have been randomized in order for an IA to be conducted by an independent DMC after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO. The Interim Analysis Set (IAS; see Section 9.7) will be used for the analyses conducted at the IA. The IA will be used to drop one or more doses of volixibat, or to terminate the trial.

Decisions about whether or not to continue randomizing to a particular dose following the 24 week IA should be made in accord with Figure 2 and its associated decision rules. In the figure, a net positive benefit risk assessment implies that the drug is more effective than any negative safety concerns. Effectively, randomization should be discontinued to any dose determined to be intolerable or to have a net negative benefit risk. Hepatic fat fraction as measured by MRI is abbreviated HFF in the figure. The liver enzyme alanine aminotransferase is abbreviated ALT.

Figure 2: Process Flow for Volixibat Dose Decisions at 24 Week IA



Decision Rules:

If randomization is discontinued to at least one dose, then randomize equally to the remaining volixibat dose(s) and PBO. That is, if two doses are determined to be tolerable and to have a net positive benefit risk assessment, then randomization to those two doses (and PBO) should continue. Further, if only one dose is determined to be tolerable and to have a net positive benefit risk assessment, then randomization to only that dose (and PBO) should continue.

Otherwise, the choice of doses to which randomization should continue should be made according to the following general principles and specific rules which are embodied in Appendix 6.

Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. That is, subjects receiving a dose to which randomization is stopped at the IA will continue to receive that same dose throughout the course of the study, and complete all required assessments. Subjects recruited in the study after completion of the IA will be randomized evenly to the remaining dose groups or PBO group by IRT. Subjects will be randomized to at most two volixibat doses as well as to PBO. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of IP.

9.5.1 Tolerability

Tolerability will be assessed by the number of discontinuations due to any one TEAE. The most common causes of discontinuation seen in healthy human trials were gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Thus, calculations used to determine dropping one or more doses of volixibat in this study were based upon the rate of evacuations seen in the Phase 1 trial SHP626-101, as this TEAE was felt to be most objectively measured. The average rate of evacuations was ~ 1.27 per subject per day for PBO. The lowest rate of evacuations on volixibat occurred on 10 mg, and was ~ 1.64 per subject per day. If this frequency of evacuations translates to average numbers of discontinuations, a difference of 6 or more discontinuations on volixibat when compared to PBO would occur by chance with less than 1% probability. Consequently, if 6 or more additional subjects treated with a specific dose of volixibat discontinue due to any particular TEAE when compared to PBO (absolute difference) then randomization to that dose will be stopped. A particular volixibat dose will be considered tolerable if fewer than 6 additional subjects discontinue due to any TEAE when compared to PBO. If none of the doses are tolerated, the study will be terminated.

9.5.2 Efficacy

Efficacy at the IA will be based on reduction of steatosis (as measured change from Baseline in HFF on MRI). If a volixibat dose does not achieve efficacy as measured by HFF reduction on MRI, percent change from Baseline in ALT at Week 24 will then be evaluated.

The sample size of 80 subjects provides 80% power to test for a net difference in HFF (volixibat dose minus PBO) of 5% at the two-sided 10% significance level. The net difference of 5% was based on the colesevelam clinical trial (Davidson et al. 2010). The statistical method used to test reduction in HFF will be an ANCOVA model which includes change from Baseline as the dependent variable, and treatment and baseline HFF as independent variables. Treatment will be a class variable and baseline HFF will be continuous. Each volixibat dose will be separately compared to PBO to assess whether its net reduction is significantly different from zero at the 10% significance level. A Holm multiplicity adjustment will be applied. If the dose is significantly different from PBO, then that dose will be considered effective at the interim assessment.

If a volixibat dose does not achieve efficacy as measured by HFF reduction on MRI, percent change from Baseline in ALT at 24 weeks will be evaluated. If the difference is at least 20% when compared to PBO, then that dose will be considered effective at the interim assessment. In

addition, if multiple doses exhibit a difference of a least 20% when compared to PBO then the statistical method used to test reduction in ALT will be an ANCOVA model which includes change from Baseline as the dependent variable, and treatment and baseline ALT as independent variables. Treatment will be a class variable and baseline ALT will be continuous. Each volixibat dose will be separately compared to PBO to assess whether its net reduction is significantly different from zero at the 10% significance level. A Holm multiplicity adjustment will be applied. If the dose is significantly different from PBO, then that dose will be considered effective at the interim assessment.

Data Monitoring Committee

An independent DMC will be established to assess safety, tolerability and efficacy during the study, as well as to ensure the validity and scientific merit of the trial. In addition, the DMC will evaluate efficacy of different doses of volixibat at the 24 week IA (using the process flow and decision rules specified previously). Based on their evaluation, the DMC will make recommendations concerning study discontinuation due to intolerability or futility, or the continuation of one or more dose groups to the 48 week liver biopsy. The DMC will monitor ongoing data generated by the study at regular intervals for the duration of the study. Their role is to protect the interests of the subjects in the study and of those still to be entered, by review of accumulating data generated in the study.

The roles, responsibilities and rules governing operation of the DMC will be discussed in full in a DMC charter. The DMC charter will define the primary responsibilities of the DMC; guide its activities, its relationship with other study components, its membership, and the purpose and timings of its meetings. It will provide the procedures for ensuring confidentiality, formal communication, and outline of the content of reports that will be provided by the DMC. Data provided to the DMC will not be considered ‘clean’ until the database is locked.

Appropriate summary statistics and data listings will be provided to the DMC by an independent statistician supported by an independent statistical reporting group not otherwise assigned to the study.

The recommendations made by the DMC to alter the conduct of the study will be forwarded to Shire for final decision. Shire will forward such decisions to regulatory authorities, as appropriate.

9.6 Sample Size Calculation and Power Considerations

These are the primary hypotheses that are being tested in this study:

- Null: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS does not differ between any of the volixbat doses and PBO.

- Alternative: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS does differ between at least one volixbat dose and PBO.

Response rates of 21% (PBO) and 45% (active) were reported in the FLINT trial (Neuschwander-Tetri et al. 2015) using the same primary end-point of reduction of NAS by at least two points (without worsening of fibrosis). Based on those response rates, 67 subjects per group completing the trial are needed for 80% power with a 10% type I error for a study comparing two volixibat doses to PBO. Holm multiplicity adjustment was included in the sample size estimate.

Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 PBO) a 24-week IA will eliminate at least one dose group based on the criteria provided to the DMC; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue. Fewer subjects are needed if more than one SHP626 dose is dropped.

9.7 Study Population

The Screened Set will consist of all subjects who have signed an informed consent.

The Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-Baseline safety assessment (eg, coming back for any visit, reporting of an AE or reporting the absence of AEs).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have a valid scored liver biopsy.

The IAS will consist of all subjects in the Safety Set who have a valid post-baseline hepatic fat fraction assessed by MRI and a valid post-baseline ALT at 24 weeks.

9.8 Efficacy Analyses

All efficacy analyses will be based on the FAS and all statistical tests will be 2-sided hypothesis tests performed at the 10% level of significance. Also, all confidence intervals will be 2-sided confidence intervals, unless otherwise stated.

9.8.1 Primary Efficacy Endpoint

The primary endpoint is the binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction, of at least 2 points without worsening of fibrosis, from baseline NAS.

The FAS will be used to assess the primary efficacy endpoint. The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS will be analyzed. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of T2DM at baseline and baseline NAS separated into two groups (NAS={4, 5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level.

Sensitivity analyses may also be conducted in addition to the primary analysis. If the proportion of subjects missing the Week 48 liver biopsy reading does not exceed 10%, then no sensitivity analyses will be conducted for the primary efficacy endpoint. Otherwise, analyses which explore the impact of the missing data on the primary efficacy endpoint will be conducted. These sensitivity analyses may compare imputations of the missing values which favor the PBO and/or imputations which favor the active. Further, if the pattern of missing values does not appear uniformly distributed among the treatment arms, imputation method(s) based on informative missingness may also be performed. The sensitivity analyses will be detailed in the Statistical Analysis Plan.

9.8.2 Secondary Efficacy Endpoints

- Change from Baseline to Week 48 in safety and tolerability of volixibat as measured by the number of subjects discontinuing treatment due to any one treatment-emergent adverse event (TEAE).

- Change from Baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).

- Change from Baseline to Week 48 on hepatic steatosis as measured by MRI-HFF for subjects included in the IA.

- Change from Baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH CRN)

- Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at Week 48.

- Change from Baseline to Week 48 on serum liver-related biochemistry as measured by:

    - alanine aminotransferase (ALT)

    - aspartate aminotransferase (AST)

    - alkaline phosphatase (ALP)

    - gamma glutamyl transferase (GGT)

    - total bilirubin (TB)

- Change from Baseline to Week 48 on metabolic indicators as measured by:
    - fasting serum glucose levels
    - insulin levels
    - HbA1c
- Change from Baseline to Week 48 on serum lipids measured by:
    - fasting total cholesterol
    - HDL-C
    - LDL-C
    - triglycerides

Descriptive statistics for all secondary endpoints will be presented for each time point at which the variable is measured (see Table 1: Schedule of Assessments).

9.8.3 Exploratory Efficacy Endpoints

- Change from Baseline to Week 48 on liver histology as measured by the SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F)
- Change from Baseline to Week 48 on anthropomorphic measures:
    - body weight
    - BMI
    - waist circumference
    - waist-hip ratio
- Change from Baseline to Week 48 in subjects with T2DM on homeostasis measured by:
    - Homeostasis model assessment 2-IR (HOMA2-IR)
    - HOMA2-beta cell function (HOMA2-%B)
- Change from Baseline to Week 48 on the EQ-5D index, a patient-reported-measure of HRQL and health status.
- Change from Baseline to Week 48 on the EQ-5D VAS score.

- Change from Baseline to Week 48 in the proportion of subjects reporting having problems (no problems, slight problems, moderate problems, severe problems, extreme problems) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire

Descriptive statistics for all exploratory endpoints will be presented for each time point at which the variable is measured (see Table 1: Schedule of Assessments).

9.9 Safety Analyses

The Safety Set will be used to assess the safety endpoints.

The number of discontinuations due to any 1 TEAE will be summarized for each treatment group.

The number of events, incidence, and percentage of TEAEs will be presented for each treatment group by system organ class, and by the preferred term using the agreed upon version of the Medical Dictionary for Regulatory Activities.

Treatment-emergent AEs will be further summarized by severity.

Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will also be summarized.

Vital signs, ECG findings, and clinical laboratory tests will be summarized for each treatment group. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. The number of events, incidence, and percentage of TEAEs will be calculated overall, by SOC, by preferred term, and by treatment group. Treatment-emergent adverse events will be further summarized by severity and relationship to the IP. Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized for each treatment group. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

9.10 Other Analyses

Serum samples to be stored.

9.10.1 Health-related Quality of Life Analyses

- Change from Baseline to Week 48 on the EQ-5D index (Appendix 3). The EQ-5D index will be derived using published UK-based preference weights. Univariate descriptive

statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from Baseline to Week 48 on the EQ-5D VAS score. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from Baseline to Week 48 in the proportion of subjects reporting having problems (none, slight, moderate, severe, extreme) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire. Proportions and 95% confidence intervals will also be generated for the baseline and Week 48 values as well as for change from baseline.

9.10.2 Stool Assessment

Stool hardness and number of evacuations will be assessed throughout the study. Stool hardness will be assessed for the softest evacuation within 24 hours of each clinic visit using the Bristol Stool Chart, a medical aid designed to classify the form of human feces into 7 categories where Type 1 is the hardest and Type 7 is the softest. Appendix 7 provides a sample of the Bristol Stool Chart. Number of evacuations within the past 24 hours prior to the clinic visit will be recorded at specified times as per Table 1.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements.

The name and address of each third party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of the IP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

10.1.3 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the

Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

10.1.5 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.do

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any co-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all the IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

eCRFs are supplied by the sponsor and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. eCRFs must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

All data sent to the sponsor must be endorsed by the investigator.

The CRA/study monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, subject diary cards, original clinical laboratory reports, and histology and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file,

appointment books, original laboratory reports, images, etc.). Non-study site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other USA national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in the IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB/EC and which received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the

sponsor, prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an EC opinion can be the sponsor, the investigator, or for multicenter studies the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the Sponsor or its designee has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor, the investigator or for multicenter studies the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All USA-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the Sponsor or its designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market volixibat; national or local regulatory authorities; and the IRB(s)/EC(s) which gave

approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results / Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee, which is done for large, multicenter Phase 2-4 and certain other studies as determined by Shire. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 60 days prior to

submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

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12. APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	24 February 2016	Global
Amendment 1	8 April 2016	Global

APPENDIX 2 FATTY LIVER DISEASE HISTOLOGY SCORING

Diagnosis: NASH, Suspicious/Borderline NASH (type a or b), NAFLD, Not NAFLD

NAS SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Lobular Inflammation	0	no foci
		1	< 2 foci per 200x field
		2	2-4 foci per 200x field
		3	> 4 foci per 200 x field
	Ballooning	0	None
		1	Rare or diagnostically borderline
		2	Many or Prominent ballooned hepatocytes

SAF SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Ballooning	0	normal hepatocytes with cuboidal shape and pink cytoplasm
		1	clusters of hepatocytes with rounded shape and pale cytoplasm usually reticulated
		2	grade 1 plus enlarged hepatocytes, 2 x bigger than normal cells.
	Lobular Inflammation	0	none
		1	< 2 foci per 200x field
		2	>2 foci per 200x field

Steatosis 1,2,3 + Ballooning 1,2 + Lobular Inflammation 1,2 = NASH
Steatosis 1,2,3 +Ballooning 0 + Lobular Inflammation 0,1,2 = NAFLD
Steatosis 1,2,3 + Ballooning 1,2 + Lobular 0 = NAFLD
Steatosis 0 = no NAFLD

FIBROSIS SCORE	None	0
	Mild zone 3 perisinusoidal (requires trichrome)	1a
	Moderate Zone 3 perisinusoidal (visible on H&E)	1b
	Portal/periportal only	1c
	Portal, periportal and perisinusoidal	2
	Bridging	3
	Cirrhosis	4

References: Kleiner et al. 2005; Bedossa 2012

ADDITIONAL SCORING FEATURES

Expanded Balloon Score

0: None

1: Few Non classic

2: Few Classic

3. Many Classic

4: Severe Classic

Portal Inflammation

0: None

1: Minimal

2: Mild

3: More than Mild

Megamitochondria

0: None

1: Present

Acidophil Bodies

0: None or rare

1: Present

For stage 2 or greater fibrosis

Portal Predominant

Central Predominant

No predominance

Steatosis Zone

Zone 1 predominant

Zone 3 predominant

Azonal

Panacinar

Glycogenosis

None

Focal

Diffuse

APPENDIX 3 EQ-5D-5L HEALTH QUESTIONNAIRE UK SAMPLE ONLY, NOT FOR OFFICIAL USE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

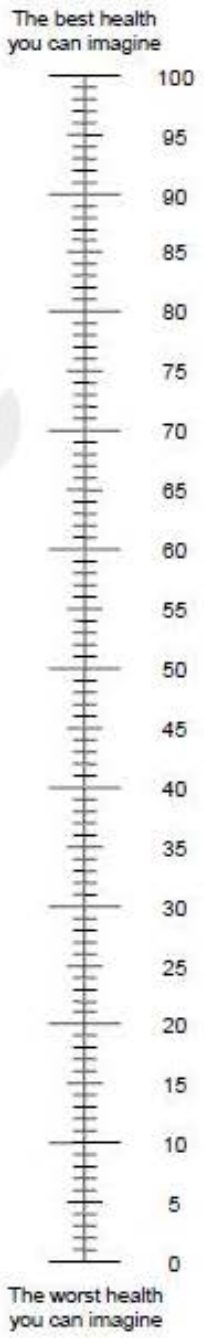
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 4 CRITERIA TO ASSESS SEVERITY OF LIVER-RELATED ADVERSE EVENTS

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
ALT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
AST	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Alkaline Phosphatase	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
GGT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Bilirubin	Normal	>1.0-1.5	>1.5-2.5	>2.5-5	>5

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
NOTE: Values expressed as multiples of the upper limit of the normal range (ULN).

APPENDIX 5 STOPPING RULES FOR LIVER-RELATED BLOOD TESTS

- ALT or AST >6xULN (confirmed with repeat within 24hrs)
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN **and** (TBL >2xULN **or** INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

If ALT or AST elevations do not fall within the above parameters, but are > 4 ×ULN subject should remain on study drug with close observation which includes:

1. Immediately contacting the CRO medical monitor.
2. Repeating liver enzyme and serum bilirubin tests two or three times weekly.

   Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
3. Obtaining a more detailed history of symptoms and prior or concurrent diseases.
4. Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
5. Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease., or other disease at the discretion of the investigator
6. Obtaining a history of exposure to environmental chemical agents.
7. Obtaining additional tests to evaluate liver function, as appropriate (eg, INR, direct bilirubin).

APPENDIX 6 GENERAL PRINCIPLES AND SPECIFIC RULES FOR DOSE DISCONTINUATION AT 24 WEEK INTERIM ANALYSIS – GUIDANCE FOR DATA MONITORING COMMITTEE ONLY

General Principles

A net positive benefit risk assessment on HFF takes precedence over effectiveness on ALT. Covers the situations when 2 doses show net positive benefit risk on HFF and 1 dose shows positive or net positive benefit risk on ALT.

Choose the dose(s) that have the largest difference in dose from amongst those exhibiting a net positive benefit risk.

As a minimally absorbed drug, volixibat at higher doses is less likely than absorbed drugs to exhibit additional TEAEs in large longer duration trials that were not seen in smaller shorter trials.

Specific Rules

If 3 doses are determined to be tolerable and to have a net positive benefit risk assessment on HFF, then Scheffe's statistical multiple comparisons test should be applied to the three ΔΔHFF values. The test will result in one of eighteen possibilities. The 18 possible cases are detailed below in Table 1. For example, if the multiple comparisons test indicates that the low dose (L) is less effective on HFF than the medium and high doses (M and H) then the medium and high doses should be selected for continued randomization from among the three doses. This case is depicted as case 1 in the table. If doses are indistinguishable on ΔΔHFF then use Scheffe's test on ΔΔALT (case 2).

Table 1 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in Hepatic Fat Fraction

Case	Results	Action
1	L < M ≅ H	M, H
2	L ≅ M ≅ H	If can't decide using ALT, then L,H
3	L > M ≅ H	L, If can't decide using ALT, then L,H
4	L ≅ M > H	L, M
5	L > M > H	L, M
6	L < M < H	M, H

Table 1 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in Hepatic Fat Fraction

Case	Results	Action
7	L ≅ M < H	H, If can't decide using ALT, then L,H
8	M < L ≅ H	L, H
9	M > L ≅ H	M, If can't decide using ALT, then H
10	M ≅ L > H	L, M
11	M > L > H	L, M
12	M < L < H	L, H
13	M ≅ L < H	H, If can't decide using ALT, then M
14	L < H ≅ M	M, H
15	L > H ≅ M	L, If can't decide using ALT, then H
16	L ≅ H > M	L, H
17	L > H > M	L, H
18	L < H < M	M, H

L = low dose (5 mg), M = medium dose (10 mg), H = high dose (20 mg)

If 3 doses are determined to be tolerable and to have a net positive benefit risk assessment on only ALT, then Scheffe's statistical multiple comparison test should be applied to the three ΔΔALT values. The test will result in one of eighteen possibilities. The 18 possible cases are detailed below in Table 2. For example, if the multiple comparison test indicates that the low dose (L), medium dose (M) and high dose (H) are indistinguishably effective on ALT, then the low and high dose should be selected. This case is depicted as case 2 in the table.

Table 2 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in ALT

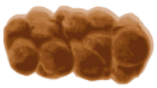
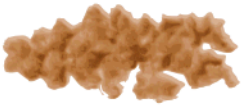
Case	Results	Action
1	L < M ≅ H	M, H
2	L ≅ M ≅ H	L,H
3	L > M ≅ H	L and H
4	L ≅ M > H	L, M
5	L > M > H	L, M
6	L < M < H	M, H
7	L ≅ M < H	H, L
8	M < L ≅ H	L, H
9	M > L ≅ H	M, H
10	M ≅ L > H	L, M
11	M > L > H	L, M
12	M < L < H	L, H
13	M ≅ L < H	H, L
14	L < H ≅ M	M, H
15	L > H ≅ M	L, H
16	L ≅ H > M	L, H
17	L > H > M	L, H
18	L < H < M	M, H

L = low dose (5 mg), M = medium dose (10 mg), H = high dose (20 mg)

If three doses are tolerable and effective and only one of them is tolerable and exhibits effectiveness on HFF, then randomization to that dose should continue. One of the remaining doses which are tolerable and effective only on ALT should be chosen based on a Scheffe's multiple comparisons test for ΔΔALT. If neither of the remaining two separates from the other with respect to ΔΔALT, then randomization should continue to the highest dose of the remaining two active doses (and PBO).

APPENDIX 7 BRISTOL STOOL CHART

Bristol Stool Chart

Type 1		Separate hard lumps, like nuts (hard to pass)
Type 2		Sausage-shaped but lumpy
Type 3		Like a sausage but with cracks on the surface
Type 4		Like a sausage or snake, smooth and soft
Type 5		Soft blobs with clear-cut edges
Type 6		Fluffy pieces with ragged edges, a mushy stool
Type 7		Watery, no solid pieces. **Entirely Liquid**

PROTOCOL: SHP626-201

TITLE:	A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)
DRUG:	Volixibat potassium (SHP626)
IND:	123,847
EUDRACT NO.:	2016-000203-82
SPONSOR:	Shire Human Genetic Therapies, Inc. 300 Shire Way Lexington, MA 02421 USA
PRINCIPAL/ COORDINATING INVESTIGATOR:	Philip Newsome MD Director of Centre for Liver Research University of Birmingham Edgbaston, Birmingham B15 2TT UK
NATIONAL/ COORDINATING INVESTIGATOR/UK	Guruprasad P Aithal MBBS, MD, FRCP, PhD Head of the Division, Nottingham Digestive Diseases Centre Director, NIHR Nottingham Digestive Diseases Biomedical Research Unit Nottingham University Hospitals and University of Nottingham Queen's Medical Centre Nottingham NG7 2UH
PROTOCOL HISTORY:	Original Protocol: 24 February 2016

PROTOCOL SIGNATURE PAGE

Sponsor's (Shire) Approval

Signature: MD FAASLD **Date:** 29 Feb 2016

Melissa Palmer, MD FAASLD
Global Clinical Development Lead- Hepatology

Investigator's Acknowledgement

I have read this protocol for Shire Study SHP626-201.

Title: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address:
(please hand print or type)

Signature: _____ **Date:** _____

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the Shire Clinical Study Serious Adverse Event and Non-serious Adverse Events (AEs) Required by the Protocol Form within 24 hours to the Shire Global Pharmacovigilance and Risk Management Department. Applicable fax numbers and e-mail address can be found on the form (sent under separate cover). A copy of this form must also be sent to the contract research organization (CRO) by fax or e-mail using the details below.

For protocol- or safety-related issues during normal business hours 8:30 a.m. to 4:30 p.m. (EST), the investigator must contact the CRO Medical Monitor:

CRO Medical Monitor: Anthony Japour, MD

telephone number: +1 215 616 6439

fax number: +1 215 616 3096

mobile number: +1 267 429 6601

e-mail address: Anthony.Japour@iconplc.com

For protocol- or safety-related issues outside of normal business hours, the investigator must contact the CRO On-call Mobile Phone:

On-call mobile number: +1 215 353 2206

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints to Shire within 24 hours. This includes any instances wherein the quality or performance of a Shire product (marketed or investigational) does not meet expectations (eg, inadequate or faulty closure, product contamination) or that the product did not meet the specifications defined in the application for the product (eg, wrong product such that the label and contents are different products). For instructions on reporting AEs related to product complaints, see Section 8.

Please use the information below as applicable to report the Product Quality Complaint:

Origin of Product Quality Complaint	**E-mail Address**
North and South America	PQC@shire.com
European Union and Rest of World	PQCROW@shire.com

Telephone numbers (provided for reference if needed):

Shire, Lexington, MA (USA)
1-888-300-6414 or 1-800-828-2088

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ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPPT	activated partial thromboplastin time
ASBT	apical sodium-dependent bile acid transporter
ASBTi	ASBT inhibitor
AST	aspartate aminotransferase
β-HCG	beta-human chorionic gonadotropin
BA	bile acid
BMI	body mass index
C4	7-alpha-hydroxy-4-cholesten-3-one
CRA	clinical research associate
CRC	clinical research center
CRO	contract research organization
DMC	data monitoring committee
eCRF	electronic case report form
EQ-5D-5L	EuroQol-5 Dimension-5 Level Questionnaire
ECG	electrocardiogram
EOS	end of study
EU	European Union
FAS	full analysis set
FBA	fecal bile acid
FDA	Food and Drug Administration
FXR	farnesoid X receptor
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
GLP1 RA	glucagon-like peptide-1 receptors agonists
HbA1c	hemoglobin A1c
HBcAb	hepatitis B core antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBVDNA	hepatitis B virus deoxyribonucleic acid
HCV	hepatitis C virus

HCVAb	hepatitis C virus antibody
HCVRNA	hepatitis C virus ribonucleic acid
HDL-C	high-density lipoprotein-cholesterol
HFD	high fat diet
HFF	hepatic fat fraction
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HOMA2-%B	homeostatic model assessment in β-cell function
HOMA2-IR	homeostatic model assessment insulin resistance
HRQoL	health-related quality of life
IA	interim analysis
IAS	interim analysis set
ICH	International Conference on Harmonisation
IND	investigational new drug
INR	international normalized ratio
IP	investigational product
IR	insulin resistance
IRB	Institutional Review Board
IRT	interactive response technology
LDL-C	low-density lipoprotein-cholesterol
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MedDRA	Medical Dictionary for Regulatory Activities
MR	magnetic resonance
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MOA	mechanism of action
NAFLD	nonalcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	nonalcoholic steatohepatitis
NASH CRN	NASH Clinical Research Network
NOAEL	no observed adverse effect level
PBC	primary biliary cirrhosis
PBO	placebo
PDFF	proton density fat-fraction

PO	by mouth
POC	proof of concept
PRO	patient-reported outcome
PSC	primary sclerosing cholangitis
PTT	prothrombin time
PVRM	Pharmacovigilance and Risk Management
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAF	steatosis, activity, and fibrosis score
SAP	statistical analysis plan
T2DM	type 2 diabetes mellitus
T3	triiodothyronine
TA	therapeutic area
TB	total bilirubin
TEAE	treatment-emergent adverse event
TGR5 G	transmembrane G protein-coupled receptor
TPN	total parenteral nutrition
TSH	thyroid-stimulating hormone
TZD	thiazolidinediones
ULN	upper limit of normal
USA	United States of America
VAS	visual analogue scale

STUDY SYNOPSIS

Protocol number: SHP626-201	**Drug:** Volixibat potassium (SHP626)
Title of the study: A Phase 2 Double-Blind, Randomized, Placebo-controlled, Dose-finding Study to Evaluate the Safety, Tolerability and Efficacy of Volixibat Potassium, an Apical Sodium-Dependent Bile Acid Transporter Inhibitor (ASBTi) in Adults with Nonalcoholic Steatohepatitis (NASH)	
Number of subjects (total and for each treatment arm): Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 placebo [PBO]) a 24-week interim analysis (IA) will eliminate at least one dose group based on the criteria provided to the Data Monitoring Committee; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue. Fewer subjects are needed if more than one SHP626 dose is dropped.	
Investigator(s): Multicenter study	
Site(s) and Region(s): Anticipated regions -US, Canada, and EU Estimated number of sites planned – 60 to 80	
Study period (planned): July 2016 to July 2019	**Clinical phase:** 2
Objectives: **Primary:** To evaluate the effect of volixibat compared to PBO on liver histology **Secondary:** • To evaluate the safety and tolerability of volixibat compared to PBO • To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI) • To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual nonalcoholic fatty liver disease (NAFLD) activity score (NAS) components and fibrosis stage) • To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis) • To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry • To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c]) • To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)	
Rationale: Currently, there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its mechanism of action (MOA) and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.	
Investigational product, dose, and mode of administration: • Volixibat 5, 10, and 20 mg and matched PBO capsules by mouth (PO) once daily (QD). Investigational	

product (IP) should be given 30 minutes prior to the first meal of the day containing fat

- Identical PBO will be used as comparator
- Subjects should take the IP at the same time each day and should not take more than one dose in a day if they miss a dose

Methodology:

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

Inclusion and exclusion criteria:

Inclusion Criteria:

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally-authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first Screening visit.
4. Male, or non-pregnant, non-lactating female who is sexually active and who agrees to comply with any applicable contraceptive requirements of the protocol or females of non-childbearing potential.

   Males and females of child-bearing potential who are sexually active, or are not currently sexually active during the period of the study, but become sexually active during the study and 30 days following the last dose of the IP, must agree to use acceptable contraception.
5. Presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis from a centrally read liver biopsy performed either during the Screening period or within 6 months prior to the first visit with a NAS of ≥ 4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

Exclusion Criteria:

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV (defined as HCVAb positive without a current or prior history of a detectable HCVRNA) will be eligible, alcoholic liver disease, proven autoimmune hepatitis, primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.
3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the IP (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥ 5% after qualifying liver biopsy performed.
5. Contraindications to MRI (ie, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study, or any condition that presents undue risk from the IP or procedures.
7. Vitamin E, thiazolidinediones (TZD), and glucagon-like peptide-1 receptors agonists (GLP1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation.
8. Uncontrolled diabetes defined as HbA1c of ≥ 9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment.

(Current use is defined as use within 14 days of Screening).

10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to qualifying liver biopsy, or initiated any time after qualifying liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST > 5 times upper limit of normal (ULN) at Screening.
12. Serum ALT > 5 times ULN at Screening.
13. Elevated serum creatinine ≥ 2.0 mg/dL.
14. International normalized ratio (INR) >1.3.
15. Total bilirubin (TB) ≥ 2.0 x ULN at Screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count < 130×10^9L
17. Medical history of impaired hemostasis or use of anticoagulant medication (baby aspirin (ASA) will be allowed).
18. Uncontrolled thyroid disease.
19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year. Weekly alcohol intake greater than 21 units for males and 14 units for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
25. Known positivity for human immunodeficiency virus (HIV) infection.
26. Cancer within 5 years of Screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
27. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
28. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
29. Subject has failed Screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
30. Subjects who are employees at the investigational site.

Maximum duration of subject involvement in the study:

- Planned duration of screening period: 42 days

- Planned duration of enrollment period: 364 days
- Planned duration of treatment period: 336 days
- Planned duration of follow-up: 28 days

Endpoints and statistical analysis:

Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-Baseline safety assessment (eg, coming back for any visit, reporting of an adverse event (AE) or reporting the absence of AEs).

Full Analysis Set (FAS) will consist of all subjects in the Safety Set who have a valid scored liver biopsy.

Interim Analysis Set (IAS) will consist of all subjects in the Safety Set who have a valid post-baseline hepatic fat fraction (HFF) assessed by MRI and a valid post-baseline ALT at Week 24.

Screened Set will consist of all subjects who have signed an informed consent.

Endpoints:

- ***Primary:*** Binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS.
- ***Secondary:***
  - Change from Baseline to Week 48 in safety and tolerability of volixibat as measured by the number of subjects discontinuing treatment due to any one treatment-emergent adverse event (TEAE).
  - Change from Baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).
  - Change from Baseline to Week 48 on hepatic steatosis as measured by MRI-HFF
  - Change from Baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH Clinical Research Network (CRN) system and Ishak)
  - Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at week 48.
  - Change from Baseline to Week 48 on serum liver-related biochemistry as measured by:
    - alanine aminotransferase (ALT)
    - aspartate aminotransferase (AST)
    - alkaline phosphatase (ALP)
    - gamma glutamyl transferase (GGT)
    - total bilirubin (TB)
  - Change from Baseline to Week 48 on metabolic indicators as measured by
    - fasting serum glucose levels
    - insulin levels
    - HbA1c
  - Change from Baseline to Week 48 on serum lipids measured by
    - fasting total cholesterol
    - HDL-C
    - LDL-C
    - triglycerides

Primary hypothesis: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS differs between at least one volixibat dose and PBO.

The FAS will be used to assess the primary efficacy endpoint. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of Type 2 diabetes mellitus (T2DM) at Baseline, NAS, and fibrosis. Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level. Histology will be read by one central hepatohistopathologist who will use the NASH CRN standard scoring system – NAS. The SAF Steatosis (S),

Activity (A), and Fibrosis (F) scoring system will be determined for exploratory purposes.

The Safety Set will be used to assess the safety endpoints including AEs (including changes from Baseline in physical examination findings), vital signs, ECGs, and clinical laboratory tests (chemistry, hematology, coagulation and urinalysis).

AEs will be coded using the agreed upon version of the Medical Dictionary for Regulatory Activities (MedDRA). TEAEs are defined as AEs that started or worsened on or after the date of the dose of IP, and no later than the study completion visit (or follow-up visit for subjects who terminate the study early). The number of events, incidence, and percentage of TEAEs will be presented by system organ class and preferred term. TEAEs will be further summarized by severity and the relationship to the IP. AEs related to the IP, AEs leading to withdrawal, SAEs, and death will be listed. The number of discontinuations due to any 1 TEAE will be summarized for each treatment group.

Vital signs, ECG findings, and clinical laboratory tests will be listed for each subject and summarized for each treatment arm, including a flag for any potentially clinically important findings. Graphical presentation may be used when deemed necessary.

For safety parameters, baseline is defined as the last assessment prior to the dose of the IP.

Planned Interim Analysis:

An IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO, on reduction of steatosis as measured by HFF by MRI and ALT.

Tolerability will be assessed by the number of discontinuations due to any one TEAE, presumably gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Efficacy at the IA will be based on reduction of steatosis or ALT. Steatosis is assessed by MRI-HFF. Depending on the results from the IA, one or more doses of volixibat may be discontinued or the study may be terminated. If the study is not terminated, subjects will receive a total of 48 weeks of the IP.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-6 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-42 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
Informed consent	X									
Inclusion/exclusion criteria	X	X (review)								
Demography, medical & medication history	X									
Physical examination	X	X					X		X	X
Height[c], weight[d], waist circumference and waist:hip ratio	X	X	X	X	X	X	X	X	X	X
Vital signs[e]	X	X	X	X	X	X	X	X	X	X
PRO (EQ-5D-5L)		X					X		X	
Liver biopsy[f]	X								X	
MRI[g]	X						X[h]		X[h]	
ECG (12-lead)[i]	X	X				X			X	
Biochemistry and Hematology[j]	X	X	X	X	X	X	X	X	X	X
Serum Glucose[j,k]	X	X	X	X	X	X	X	X	X	X
Urinalysis[l]	X	X							X	
Urine Drug and Serum Alcohol Tests	X	X								
Urine Pregnancy Test[m]	X	X	X	X	X	X	X	X	X	X
Lipid Panel[j,n]	X	X	X	X	X	X	X	X	X	X
Coagulation Panel[j,o]	X	X		X			X		X	
Vitamin D[j]	X	X		X			X		X	

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	**1**	**2**	**3**	**4**	**5**	**6**	**7 IA**	**8**	**9 EOS**	**10 In-Clinic**
Week	**-6 to 0**	**0**	**2**	**4**	**8**	**12**	**24**	**36**	**48**	**52**
Study Day	**-42 to -1**	**0**	**14**	**28**	**56**	**84**	**168**	**252**	**336**	**364**
HbA1c[j]	X	X	X	X	X	X	X	X	X	X
Serum Liver-Related Blood Tests[j,p]	X	X	X	X	X	X	X	X	X	X
Insulin[j]	X	X		X		X	X	X	X	X
HIV, Hepatitis B/C[j,q]	X									
Thyroid testing[j,r]	X	X		X		X	X		X	X
C4 Sampling		X				X	X		X	X
IRT Accessed	X	X	X	X	X	X	X	X	X	X
Randomization		X								
IP Dispensed[s]		X	X	X	X	X	X	X		
IP Returned/Accountability & Compliance Assessed			X	X	X	X	X	X	X	
Stool Assessment[t]		X	X			X		X	X	X
Adverse Events[u]	X	X	X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X	X

ECG = electrocardiogram; EOS = end of study; HOMA = Homeostasis Model Assessment; IA = interim analysis; IP = investigational product; IRT = Interactive Response Technology; MRI = magnetic resonance imaging; PRO = patient-reported outcome.

[a] Visit Windows (calculated from Visit 2): Bi-weekly (Visits 3-4): +/- 3 days; Monthly (Visits 5-6): +/-5 days; Tri-monthly (Visits 7-9/DC): +/- 7 days.

[b] Subjects will be reminded not to eat prior to their scheduled visit. Additionally, during the double-blind treatment period, they should not take their study drug prior to the visit. They should bring their study drug with them to the visit to take 30 minutes prior to their first meal of the day containing fat.

[c] Height to be measured at Screening only.

[d] BMI to be calculated programmatically by the Sponsor or designee for the following visits: Screening (Visit 1), Baseline (Visit 2), Visits 7 and 9/DC.

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9 EOS	10 In-Clinic
Week	-6 to 0	0	2	4	8	12	24	36	48	52
Study Day	-42 to -1	0	14	28	56	84	168	252	336	364

[e] Vital signs to include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate.

[f] Biopsy performed within 6 months of Screening can be used. All biopsies will be centrally read by a hepatic histopathologist.

[g] MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.

[h] MRI at Visit 7 (Week 24) and at Visit 9 (Week 48) for the Interim Analysis Set only.

[i] Three ECGs will be recorded with approximately 10 minutes between each ECG for the Baseline evaluation.

[j] All blood tests are fasting blood tests.

[k] HOMA2-IR and HOMA2-%B: will be calculated programmatically by the Sponsor or designee for the following visits: Screening (Visit 1), Baseline (Visit 2), Visits 7, 9/DC, and 10.

[l] Urinalysis to include oxalate testing.

[m] For all females of child-bearing potential (FOCP). Positive on-site urine dipstick results must have serum reflex testing performed by central lab. Additional testing can be performed at the investigator's discretion.

[n] Lipid Panel includes fasting total cholesterol, HDL-C, LDL-C, and triglycerides.

[o] Full coagulation panel will be done at Screening and Baseline, but only INR and PT are required at remaining time points.

[p] Serum Liver-Related Blood Tests include ALT, AST, ALP, GGT, and total bilirubin.

[q] Hepatitis B/C testing includes HBcAb, HBsAg, HBVDNA and HCVAb, HCVRNA, respectively.

[r] Thyroid testing includes thyroid stimulating hormone (TSH) and triiodothyronine (T3).

[s] Investigational product may be dispensed at an unscheduled visit outside of this schedule as needed to replace lost or damaged product.

[t] Subjects will be queried about the number of stool evacuations during the 24- hour period before the clinical research center (CRC) visit and asked to describe the consistency of the softest stool during that 24- hour period using the Bristol Stool Chart.

[u] Adverse events will be collected beginning from the signing of informed consent. All AEs must be followed to closure (the subject's health has returned to his/her Baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise

Table 1: Schedule of Assessments

Period	Screening	Baseline	Double-blind Treatment							Follow-up
Visit[a, b]	1	2	3	4	5	6	7 IA	8	9 EOS	10 In-Clinic
Week	-6 to 0	0	2	4	8	12	24	36	48	52
Study Day	-42 to -1	0	14	28	56	84	168	252	336	364
explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.										

1. BACKGROUND INFORMATION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide (Vernon et al. 2011), and is estimated to occur in 30-40% of adults in the United States and up to 30% of European adults. These numbers approach 95% in those with morbid obesity (Mathus-Vliegen et al. 2012). NAFLD ranges from simple steatosis, which is typically nonprogressive, to nonalcoholic steatohepatitis (NASH), which has a 20% likelihood of progression to advanced disease including fibrosis, cirrhosis and its complications including liver failure and the need for a liver transplant (Angulo 2002). Hepatocellular carcinoma is also a complication of NASH that may occur with or without the presence of cirrhosis (Torres et al. 2012). NAFLD is typically associated with type 2 diabetes mellitus (T2DM) (Adibi et al. 2007; Loomba et al. 2012), central or visceral obesity (Souza et al. 2012), dyslipidemia (Assy et al. 2000; N.C.E.P. 2002), and hypertension (Donati et al. 2004). Together these conditions comprise the metabolic syndrome, and NASH is considered to be the hepatic component of this syndrome (Hamaguchi et al. 2005, Neuschwander-Tetri 2005, Marchesini et al. 2005). Notably, NAFLD is a clinical condition occurring in individuals who do not drink excessive alcohol (>20 grams/day), yet have hepatic histology which is indistinguishable from that seen with alcoholic excess. The pathophysiology of NASH is likely multifactorial and may include combinations of metabolic, genetic, environmental, and gut microbial factors.

Most individuals with NASH are asymptomatic or have nonspecific symptoms such as fatigue. They typically first come to medical attention incidentally following routine blood testing or on imaging studies performed routinely or during the evaluation of an unrelated condition. While ultrasound and magnetic resonance imaging (MRI) can detect the presence of steatosis (Reeder et al. 2011), a liver biopsy is required to diagnose NASH and the extent of liver fibrosis.

1.1 Indication and Current Treatment Options

There are currently no drugs approved for the treatment of NASH and it is estimated that there are between 6-16 million people in the United States with NASH, of which 600,000 have severe disease (Williams et al. 2011 and Torres and Harrison 2008), with similar percentages reported throughout most areas of the world (World Gastroenterology Organisation 2012). Treatment of associated metabolic comorbidities, weight reduction, and incorporation of an exercise routine remain the cornerstone of management. However, lifestyle changes are seldom successful. Thus, NASH represents a disease with an unmet medical need that is growing at an epidemic rate, and that if untreated, carries a risk of significant morbidity and mortality.

1.2 Product Background and Clinical Information

Volixibat potassium (SHP626; formerly LUM002), hereafter referred to as volixibat, is a highly selective inhibitor of the apical sodium-dependent bile acid transporter (ASBT) that is being evaluated for the treatment of NASH.

Bile acids (BA) promote bile flow, activation of digestive enzymes, and micellization of fats and fat-soluble vitamins, thereby permitting their intestinal absorption. BAs serve as signaling molecules acting via receptors, such as farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR5 G), in the intestine, liver and other tissues which play an important role in regulating insulin homeostasis and stimulating the release of fibroblast growth factors in the liver (Halilbasic et al. 2013). Lipid peroxidation and oxidant stress have been proposed as one link between the accumulation of fat and subsequent injury (Day and James 1998). All of these metabolic actions have important effects to prevent the ongoing liver damage in NASH.

High fat diet (HFD) fed mice treated with an ASBT inhibitor (ASBTi) (SC-435 a surrogate SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved insulin resistance (IR), and decreased NAS, predominantly steatosis. In addition the BA pool reflected increases in BA that were agonists to FXR and decreases in levels that were antagonist to the FXR (Rao et al. 2015). Miethke and colleagues (2016) found that pharmacological inhibition of the ileal, ASBT (SC-435 the surrogate molecule of volixibat), blocked progression of sclerosing cholangitis in mdr2–/– mice. Beneficial effects in liver histology in these mice included a reduction in the severity of hepatic fibrosis, a decrease which correlated with a reduction of hepatic profibrogenic gene expression. While very encouraging, whether or not the findings in this mouse sclerosing cholangitis model can translate to histologic improvements in patients with NASH is unknown.

Volixibat, as a potent inhibitor of ASBT, increases BA excretion and facilitates signaling in the intestine that regulates serum and hepatic BA concentrations, glucose metabolism, serum cholesterol and fatty acid metabolism in the liver. The combined events resulting from inhibiting BA reuptake are hypothesized to have a positive metabolic, anti-inflammatory, anti-steatotic, and potentially antifibrotic effect that will lead to a therapeutic benefit for patients with NASH.

One of the factors contributing to the pathogenesis of NASH is abnormal cholesterol metabolism and the accumulation of free cholesterol in the liver. The free cholesterol is directly toxic to hepatocytes, which leads to inflammation and fibrosis (Musso et al. 2013). One treatment approach is to remove the cholesterol from the liver to decrease and possibly reverse the damage to the hepatocytes. This is one of the mechanisms by which SHP626 will treat NASH.

Due to the mechanism of action (MOA), volixibat is under development for the treatment of NASH and may also be able to improve the metabolic syndrome that is associated with NASH. Volixibat inhibits ASBT therefore BAs are excreted in the feces and this loss forces the liver to synthesize new BA which utilizes cholesterol in the liver and serum. Volixibat also has the potential to reduce IR, which is considered to be the most common underlying risk factor for the development of NASH (Pagano et al. 2002; Sanyal et al. 2001).

Volixibat was initially being evaluated for its use as an intervention for dyslipidemia. As a result of initial observations in animals that serum low-density lipoprotein cholesterol (LDL-C) and total liver cholesterol content could be decreased after administration of volixibat, additional work was undertaken to support the safety and tolerability of the drug in healthy volunteers.

Clinical and non-clinical studies have demonstrated very low systemic exposure across species. Oral administration of volixibat at doses up to 300 mg once daily and 50 mg administered daily for 14 days to healthy male subjects was generally safe. A Phase 1 study revealed that volixibat is tolerated at the 10 mg dose for 28 days and that there were trends towards increasing high-density lipoprotein cholesterol (HDL-C), decreasing LDL-C, and decreasing fasting glucose in patients with T2DM (LUM002-101 trial).

A healthy volunteer study investigating 12 days of varying levels of repeat doses of volixibat has provided key pharmacodynamic information for dose selection as measured by the effect of volixibat on excreted fecal bile acid (FBA) levels (SHP626-101). In addition, the study investigated safety and tolerability over the dose range that was considered for this Phase 2 study.

Data from Phase 1 studies support the position that volixibat is basically a non-absorbed drug that works locally in the GI tract and results in virtually no systemic exposure. Thus pharmacokinetic sampling will not be done in this study.

The current proof of concept (POC) Phase 2 trial will evaluate the safety, tolerability, and efficacy of three doses of volixibat (5, 10, and 20 mg) in adult subjects with NASH. Due to the MOA of volixibat, efficacy will be assessed at an interim analysis (IA) by a change of steatosis from Baseline to Week 24 compared to placebo (PBO). While the gold standard for quantification of steatosis has historically been an invasive liver biopsy, quantitative magnetic resonance (MR) imaging–based biomarkers for liver fat have evolved rapidly over the last decade, and are increasingly being incorporated into NASH clinical trials. Both MR spectroscopy (S) and MR imaging (I) proton density fat-fraction (PDFF) provide non-invasive means of quantifying intrahepatic lipid content (Reeder et al. 2012). Both techniques have been shown to be accurate, reproducible with a low degree of variability in interpretation, cost-effective and reliable biomarkers of quantitative hepatic fat (Roldan-Valadez et al. 2010) (Urdzik et al. 2012; Raptis et al. 2012). Importantly, studies demonstrate a close correlation with steatosis grade histologically (Qayyum et al. 2005; Schwenzer et al. 2009).

In a study of 51 adult subjects with NAFLD, PDFF correlated well with the grade of histologic steatosis, as the mean fat-fraction values of 8.9%, 16.3%, and 25.0% corresponded to histologic steatosis grades 1, 2, and 3, respectively ($P < .0001$) (Permutt et al. 2012). Thus, MRI will be utilized to evaluate the degree of steatosis change from Baseline in this study during the IA. The Mozart trial was a randomized, double-blind, PBO-controlled trial of 50 patients with NASH who were randomized to 24 weeks of either ezetimibe or PBO, evaluating the reduction of liver fat by MRI-PDFF as well as by histology. Results revealed that compared to histologic non-responders, histologic responders, defined as a two-point reduction in NAFLD activity score (NAS) without worsening fibrosis, had a statistically significant reduction in net MRI-PDFF of -4.1% ± 4.9 vs. +0.6% ± 4.1 ($P < 0.036$) with a mean percent change of -29.3% ± 33.0 vs. +2.0% ± 24.0 ($P < 0.004$), respectively (Loomba et al. 2015). Thus, in the current trial during the IA, an active dose will be considered to be efficacious if a ≥ 5% steatosis reduction compared to PBO is seen after 24 weeks of therapy.

Intrahepatic lipid content of less than 1% is considered to be within the normal range (Springer et al. 2015), however, from a study of 2349 people in a general population undergoing MRS, it was concluded that a PDFF value of 5.56% represented the upper limit of the normal range, as determined from the 95th percentile of PDFF in 345 individuals who were not at increased risk for hepatic steatosis (Szczepaniak et al. 2005). Thus, in the current trial, similar to other NASH trials utilizing MR for evaluation, an MRI ≥5% steatosis will be used as an inclusion criterion (Loomba et al. 2015).

1.3 Benefits and Risks

By virtue of volixibat's ability to inhibit ASBT bile acid reabsorption, there is an increase in BA excretion and signaling in the intestine that results in improvements in glucose metabolism and changes in cholesterol and fatty acid synthesis in the liver. Recently, HFD fed mice treated with an ASBTi (SC435 a surrogate of SHP626) normalized hepatic triglycerides and serum cholesterol, significantly improved IR, and decreased NAS (predominantly steatosis). In addition, these HFD-fed mice did not gain weight when treated with SC435, in spite of consuming increased calories. Finally, the BA pool in these mice changed to predominantly FXR agonist (Rao et al. 2015).

These metabolic actions and preclinical results may prove to be clinically relevant to subjects with NASH.

NASH has recently received considerable attention as awareness of the problem of liver damage and prevalence of the disorder has increased, paralleling the obesity epidemic. Consequences of liver damage are detrimental and can lead to liver failure, hepatocellular carcinoma, and the need for liver transplantation. There is no currently approved medical therapy for NASH. The large unmet medical need and the increased medical resource burden have led to the search for potential therapies to treat NASH.

Nonclinical testing established that the no observed adverse effect level (NOAEL) for volixibat in rats and dogs following 13 weeks of once-daily administration were 1000 and 500 mg/kg/day, respectively. Similarly, testing confirmed that the NOAEL for volixibat in a 6-month study in rats and a 9-month study in dogs were 1000 and 500 mg/kg/day, respectively. In both cases, these were the highest doses tested. Genotoxicity testing has yielded negative findings.

Volixibat is minimally absorbed. The pharmacokinetic profiles performed in clinical studies completed to date repeatedly suggest negligible systemic exposure. Furthermore, there has been no observation of clinically relevant changes in fat absorption parameters such as those related to fat-soluble vitamins.

The most frequent TEAEs in the Phase 1 studies were GI and were considered mechanism-based due to elevated BA concentrations in the colon. The percentage of subjects reporting at least 1 TEAE in the GI disorders SOC generally increased with an increasing volixibat dose level (Part

1 Study TDU10632 and Study LUM002-101). Most TEAEs were mild in intensity, and none were assessed as severe.

In the multiple dose studies, the most commonly reported TEAEs in subjects (both healthy and with T2DM) who received volixibat for the longest duration of 28 days in Study LUM002-101 included diarrhea and abdominal pain. The most commonly reported TEAEs in subjects receiving 50 mg volixibat for 14 days (part 3 Study TDR10633) were diarrhea and GI pain.

Overall, there were 2 SAEs (ALT increased and retinal detachment), both of which led to the discontinuation of volixibat. In part 3 of the initial Phase 1 study (TDU10633), 1 subject dosed with 50 mg volixibat for 13 days was withdrawn from the study due to a mild TEAE (which became a SAE due to prolonged hospitalization) of ALT increased that was considered related to volixibat . The subject's ALT level returned to normal after discontinuation of volixibat. A second subject dosed with 10 mg volixibat for 12 days in Study LUM002-101 reported a moderate SAE of ablation of the retina with a bleed in the vitreous body of the right eye that was considered not related to volixibat.

Overall, 3 subjects, all dosed with 5 mg volixibat in Study LUM002-101, discontinued volixibat due to non-serious TEAEs: 1 due to a related TEAE of mild hemorrhagic diarrhea, 1 due to an unrelated TEAE of moderate Epstein-Barr virus infection, and 1 due to mild related TEAEs of diarrhea and anal erosion.

Overall, the observed AEs attributable to volixibat have been self-limited as would be expected given the local MOA of ASBT inhibition in the terminal ileum. Generally, among subjects who experienced GI TEAEs, the events have been mild and diminished over the course of treatment.

Volixibat is a novel drug candidate, demonstrating limited systemic exposure across species with the potential to affect important metabolic pathways associated with NASH. The overall safety, tolerability, and preliminary activity of volixibat in available clinical trials suggest that further investigation is warranted and that there is a positive benefit to risk profile.

Always refer to the latest version of the Volixibat Potassium (SHP626) Investigator's Brochure for the overall risk/benefit assessment and the most accurate and current information regarding the drug metabolism, pharmacokinetics, efficacy, and safety of volixibat.

2. STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for the Study

Currently there is no approved medication for the treatment of NASH. Volixibat is under development for the treatment of NASH based on its MOA and is supported by nonclinical and Phase 1 data. This is a Phase 2, 48-week, dose-finding study to examine the efficacy, tolerability, and safety of volixibat in adults with NASH.

2.2 Study Objectives

2.2.1 Primary Objective

To evaluate the effect of volixibat compared to PBO on liver histology

2.2.2 Secondary Objectives

- To evaluate the safety and tolerability of volixibat compared to PBO
- To evaluate the effect of volixibat compared to PBO on hepatic steatosis (measured by MRI)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by individual NAS components and fibrosis stage)
- To evaluate the effect of volixibat compared to PBO on liver histology (measured by NASH resolution without worsening fibrosis)
- To evaluate the effect of volixibat compared to PBO on serum liver-related biochemistry
- To evaluate the effect of volixibat compared to PBO on metabolic indicators (glucose, insulin, hemoglobin A1c [HbA1c])
- To evaluate the effect of volixibat compared to PBO on serum lipids (cholesterol, HDL-C, LDL-C, triglycerides)

2.2.3 Exploratory Objectives

- To explore the effect of volixibat compared to PBO on liver histology (measured by individual SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F))

- To explore the effect of volixibat compared to PBO on anthropometric measures (body weight, body mass index (BMI), waist circumference and waist-hip ratio)

- To explore the effect of volixibat compared to PBO on homeostasis model assessment 2-IR (HOMA2-IR) and HOMA2-beta cell function (HOMA2-%B) in subjects with T2DM

- To explore the effect of volixibat compared to PBO on BA synthesis (7-alpha-hydroxy-4-cholesten-3-one [C4])

- To explore the effect of volixibat on patient-reported health-related quality of life (HRQoL) and overall health status.

3. STUDY DESIGN

3.1 Study Design and Flow Chart

This study will be a Phase 2, 48-week, multicenter, double-blind, randomized, PBO-controlled, parallel group, proof of concept, dose-finding study, with one IA after 24 weeks of treatment. There will be 3 active arms of volixibat (5, 10 and 20 mg) and a PBO arm. Subjects will be randomized to receive one of three doses of volixibat (5, 10, or 20 mg once daily (QD) or PBO in a 1:1:1:1 ratio such that a target of 266 subjects is achieved (221x1.2 is approximately 266). The 221 subjects include 201 subjects for the three arms analyzed at 48 weeks plus an additional 20 subjects to account for one dose dropped at the IA). Attempt will be made to perform the IA before any subject has had their 48-week post-treatment liver biopsy, although this will be dependent upon the rate of enrollment and dropout.

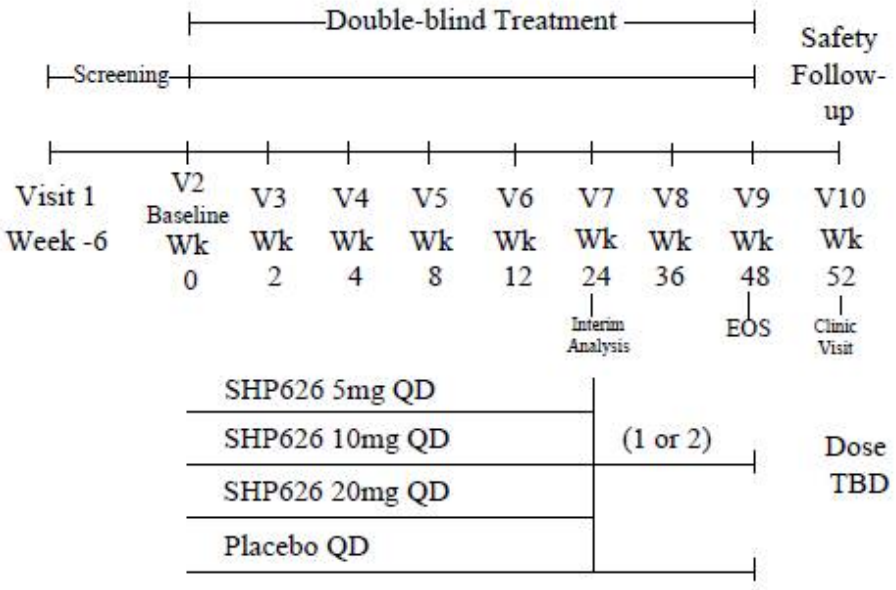
There will be up to 3 periods (Screening, Treatment and Follow-up) and an IA. The duration of the Treatment period will be 48 weeks, with an IA at Week 24. Depending on the results of the IA, the study may be terminated or the randomization to one or more doses will be stopped. The follow-up period will be 4 weeks after last dose. Subjects will be expected to visit the study center at least 10 times.

The IA will be conducted by an independent data monitoring committee (DMC) after at least 80 subjects have received 24 weeks of treatment. Study enrollment will be paused after 92 subjects have been randomized. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO, on reduction of steatosis as measured by high fat fraction (HFF) by MRI and ALT.

Once decisions as to dose elimination are made, study enrollment will be reopened and additional sites may be added. Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. Subjects recruited into the study after completion of the IA will be randomized evenly to the remaining dose groups or PBO group by IRT. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of investigational product (IP).

The study will be conducted over 3 periods: Screening (6 weeks), Treatment (48 weeks), and Follow-up (4 weeks), with an IA at 24 weeks as outlined in Figure 1.

Figure 1: Study Design Flow Chart



3.2 Duration and Study Completion Definition

The subject's maximum duration of participation is expected to be approximately 364 days. The last visit is an in-clinic safety follow-up visit. The study will be completed in approximately 3 years.

The Study Completion Date is defined as the date the final subject, across all sites, completes their final protocol-defined assessment. Please note that this includes the follow-up visit. The Study Completion Date is used to ascertain timing for study results posting and reporting.

3.3 Sites and Regions

This study will be conducted at approximately 60 to 80 clinical sites in the USA, Canada, and EU.

4. STUDY POPULATION

Each subject must participate in the informed consent process and provide written informed consent before any procedures specified in the protocol are performed.

4.1 Inclusion Criteria

The subject will not be considered eligible for the study without meeting all of the criteria below.

1. An understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via a legally authorized representative, as applicable) informed consent to participate in the study.
3. Age 18-80 years inclusive. This inclusion criterion will only be assessed at the first Screening visit.
4. Males and females of child-bearing potential who are sexually active, or are not currently sexually active during the study, but become sexually active during the period of the study and 30 days following the last dose of the IP, must agree to use acceptable contraception.

   Males and females of child-bearing potential who are not currently sexually active must agree to use acceptable contraception, if they become sexually active during the period of the study and 30 days following the last dose of the IP.
5. Presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist performed either during the Screening period or within 6 months prior to the first visit.
6. Histologic confirmation of NASH without cirrhosis from a centrally read liver biopsy performed either during the Screening period or within 6 months prior to the first visit with a NAS of ≥ 4 with a score of at least 1 in each component (steatosis, lobular inflammation, and hepatocyte ballooning).

4.2 Exclusion Criteria

Subjects are excluded from the study if any of the following exclusion criteria are met.

1. Presence of or history of cirrhosis or evidence of decompensated liver disease (ie, ascites, variceal bleeding, etc.) or hepatocellular carcinoma.
2. History or presence of other concomitant liver disease as assessed by the investigator or determined by laboratory findings including, but not limited to: active hepatitis B virus (HBV) infection (hepatitis B surface antigen (HBsAg) positive and/or HBVDNA positive; subjects who are hepatitis B core antibody (HBcAb) positive may be eligible as long as HBsAg is negative and HBVDNA is nondetectable), active hepatitis C virus (HCV) infection (prior exposure to HCV (defined as HCVAb positive without a current or prior history of a detectable HCVRNA) may be eligible alcoholic liver disease, proven autoimmune hepatitis,

primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, bile duct obstruction, liver primary or metastatic cancer.

3. Current or recurrent disease that could affect the action, absorption, disposition, or laboratory assessment of the investigational product (IP) (including bile salt metabolism in the intestine) eg, uncontrolled inflammatory bowel disease, uncontrolled celiac disease, gastric bypass procedures (gastric lap band is acceptable), ileal or ileocecal resection, uncontrolled irritable bowel syndrome with predominant diarrhea, or history of chronic diarrhea or loose stools of any etiology.
4. Weight change ≥ 5% after qualifying liver biopsy performed.
5. Contraindications to MRI (eg, claustrophobia, coronary stents, coronary implantable devices, girth, etc.).
6. Current or relevant history of physical or psychiatric illness, any medical disorder that may require treatment or make the subject unlikely to complete the study, or any condition that presents undue risk from the IP or procedures.
7. Vitamin E, thioglitazones, and glucagon-like peptide-1 receptors agonists (GLP1 RA) unless subject on a stable dose for 6 months prior to qualifying liver biopsy and not initiated after qualifying liver biopsy and will continue the same dosing regimen throughout study participation.
8. Uncontrolled diabetes defined as HbA1c of ≥ 9.0% within 60 days prior to enrollment.
9. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 14 days of Screening). Patients currently taking insulin will not be excluded; however, they must be on a stable dose for at least 30 days prior to Screening.
10. Use of drugs, herbs or supplements historically associated with causing or worsening NAFLD/NASH for less than 6 months prior to liver biopsy, or initiated any time after liver biopsy performed, including the use of total parenteral nutrition (TPN).
11. Serum AST > 5 times upper limit of normal (ULN) at Screening.
12. Serum ALT > 5 times ULN at Screening.
13. Elevated serum creatinine ≥ 2.0 mg/dL.
14. International normalized ratio (INR) >1.3
15. TB ≥ 2.0 x ULN at Screening (Except for documented Gilbert's syndrome with bilirubin levels 20 µmol/L to 90 µmol/L (1.2 to 5.3 mg/dL) and with a ratio of unconjugated/conjugated bilirubin that is commensurately higher).
16. Platelet count < 130×10^9/L
17. Medical history of impaired hemostasis or use of anticoagulant medication (baby aspirin (ASA) will be allowed).
18. Uncontrolled thyroid disease.

19. Type 1 diabetes mellitus.
20. Known or suspected intolerance or hypersensitivity to the IP, closely-related compounds, or any of the stated ingredients.
21. Known history of alcohol or other substance abuse within the last year. Weekly alcohol intake greater than 21 units for males and 14 units for females on average or inability to reliably quantify alcohol consumption based on investigator's judgment.
22. Within 6 months of MRI and liver biopsy:
    - Have used any IP
    - Have been enrolled in a clinical study (including vaccine studies) that, in the investigator's opinion, may impact this Shire-sponsored study
23. Inability to safely obtain a liver biopsy.
24. The anticipated need for a surgical procedure during the study that could interfere with the treatment.
25. Known positivity for human immunodeficiency virus (HIV) infection.
26. Cancer within 5 years of Screening, except for basal or squamous cell carcinoma of the skin or in situ cervical carcinoma that has been treated with no evidence of recurrence.
27. History of noncompliance with medical regimens, unreliability, mental instability or incompetence that could compromise the validity of informed consent or lead to noncompliance with the study protocol.
28. Any other conditions or abnormalities which, in the opinion of the investigator, may compromise the safety of the subject, or interfere with the subject participating.
29. Subject has failed Screening or was previously enrolled in this study or is currently enrolled in this study at any study site (unless the subject is transferring to another qualified study site with prior Sponsor approval).
30. Subjects who are employees at the investigational site.

4.3 Restrictions

Subjects must adhere to the following restrictions for the duration of the study:

- Subjects must remain compliant with inclusion/exclusion criteria.
- Subjects should not become pregnant, father a child, or nurse/breastfeed a baby.
- Subjects should be encouraged to adhere to the same exercise routine and a healthy diet throughout the study.

4.4 Reproductive Potential

4.4.1 Female Contraception

Sexually active females of child-bearing potential should be using an acceptable form of contraception. Females of child-bearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of the IP. If hormonal contraceptives are used they should be administered according to the package insert. Females of child-bearing potential who are not currently sexually active must agree to use acceptable contraception, as defined below, if they become sexually active during the period of the study and 30 days following the last dose of the IP.

Female subjects should be either:

- Post-menopausal (12 consecutive months of spontaneous amenorrhea and age ≥ 51 years)
- Surgically sterile (having undergone one of the following surgical acts: hysterectomy, bilateral tubal ligation, bilateral oophorectomy, or bilateral salpingectomy) and at least 6 weeks post-sterilization, or
- Females of child-bearing potential with a negative urine and/or serum β-human chorionic gonadotropin (β-HCG) pregnancy test each study visit. Females of child-bearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception.

Acceptable methods of contraception are:

- Intrauterine devices plus condoms
- Double-barrier methods (eg, condoms and diaphragms with spermicidal gel or foam)
- Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring), stabilized for at least 30 days prior to the Screening visit (Visit 1), plus condoms. Note: if subject becomes sexually active during the study, they should use one of the other acceptable methods noted above in addition to the hormonal contraceptive until it has been stabilized for 30 days.

4.4.2 Male Contraception

Contraception is required for all sexually-active male subjects and their partners. All male subjects (including those who are sterile) agree not to donate sperm, and to use 1 of the following approved methods of contraception from the Baseline visit on Day 0 until 30 days following study discharge:

- Male condom with spermicide
- Sterile sexual partner
- Intrauterine device with spermicide (use by female sexual partner)
- Female condom with spermicide (use by female sexual partner)
- Contraceptive sponge with spermicide (use by female sexual partner)
- Intravaginal system (eg, NUVARING. with spermicide, a diaphragm with spermicide, a cervical cap with spermicide) (use by female sexual partner)
- Oral, implantable, transdermal, or injectable hormonal contraceptive (use by female sexual partner).

4.5 Discontinuation of Subjects

A subject may withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution. The investigator or sponsor may withdraw the subject at any time (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject from the IP with the medical monitor when possible.

If the IP is discontinued, regardless of the reason, the evaluations listed for both Visit 9/end of study (EOS) and Visit 10/Follow-up, are to be performed as completely as possible; however, the EOS liver biopsy will not be required for patients discontinuing prior to Week 44.

Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for termination, date of stopping the IP, and the total amount of the IP taken must be recorded in the electronic case report form (eCRF) and source documents.

Subjects who discontinue will not be replaced.

A subject will be withdrawn from the study if they have missed > 3 consecutive days of study drug dosing for any reason.

4.5.1 Subject Withdrawal Criteria

Medically important events that in the opinion of the investigator, medical monitor or sponsor would compromise the subject's ability to safely continue in the study may result in withdrawal of the subject from the study.

4.5.2 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's medical record and on the eCRF. If a subject is withdrawn for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be entered on the eCRF.

Reasons for discontinuation include but are not limited to:

- Adverse event (AE)
- Protocol violation
- Withdrawal by subject
- Lost to follow-up
- Lack of efficacy
- Death
- Screen failure
- Noncompliance with study
- Physician decision
- Pregnancy
- Progressive disease
- Study terminated by sponsor
- Other - If "Other" is selected, the investigator must specify on the eCRF

4.5.3 Subjects Lost to Follow-up Prior to Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject lost to follow-up at any time point prior to the last scheduled contact clinic visit. At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

4.5.4 Safety-related Stopping Rules

Refer to Appendix 4 and Appendix 5 for criteria to assess severity of liver-related AEs and stopping rules for liver-related blood tests, respectively.

An urgent safety review will be conducted within 7 days by the sponsor if one or more of the

following criteria are met:

- Death that is considered related to the study drug
- Two SAEs of similar type (defined as same or similar Medical Dictionary for Regulatory Activities (MedDRA) higher level group code), and considered related to the study drug

The urgent review will be performed by a sponsor safety review group, which will include the study Pharmacovigilance and Risk Management (PVRM) physician and the PVRM therapeutic area (TA) Head. The PVRM TA Head, not the PVRM physician involved in the study, may be unblinded as part of this urgent safety review, if required. Following the sponsor's review of safety data, one of the following actions will be taken with respect to study status:

- Continue study with protocol unchanged
- Continue study with modifications to the protocol
- Terminate study

Subject safety will be monitored on a continuous basis during this study until the last subject completes his or her last scheduled study visit/assessment.

5. PRIOR AND CONCOMITANT TREATMENT

All non-study treatment (including but not limited to herbal treatments, vitamins, behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate) received within 30 days prior to signing informed consent at the Screening visit (Visit 1) and through the final study contact (including protocol-defined follow-up period) must be recorded on the appropriate eCRF page.

5.1 Prior Treatment

Prior treatment includes all treatment (including but not limited to herbal treatments, vitamins, and behavioral treatment, non-pharmacological treatment, such as psychotherapy, as appropriate), received within 30 days of the date of first dose of the IP. Prior treatment information must be recorded on the appropriate eCRF page.

5.2 Concomitant Treatment

Concomitant treatment refers to all treatment taken between the dates of the first dose of the IP and the end of the follow-up period, inclusive. Concomitant treatment information must be recorded on the appropriate eCRF page.

5.2.1 Permitted Treatment

Medications and supplements including vitamin E, thiazolidinediones (TZDs) and GLP1 RAs that have been used to treat NAFLD/NASH are allowed during the course of the study if the subject has been on a stable dosing regimen (ie, same dose and frequency in the previous 6 months prior to the qualifying liver biopsy and not initiated after qualifying liver biopsy) and will continue this dosing regimen throughout study participation. The investigator must contact the CRO medical monitor then the sponsor medical monitor to discuss any changes to concomitant medications that may impact the study.

5.2.2 Prohibited Treatment

The following list of prohibited drugs cannot have been taken for more than 2 weeks within the year prior to randomization and are excluded while on study.

- Systemic Glucocorticoids
- Tamoxifen
- Amiodarone
- Valproate
- Nucleoside Analogues
- Tetracycline
- Estrogens at doses greater than

- Methotrexate
- Alcohol (see Section 4.2)
- Griseofulvin
- Total parenteral nutrition
- used for hormone replacement
- Anabolic steroids
- Bile acid sequestrants such as cholestyramine or colestipol
- Any other known hepatotoxins

Treatments not listed above are considered allowable, unless considered a potential hepatotoxin. Antidiarrheals will be allowed at the discretion of the investigator, with the exception of BA sequestrants.

6. INVESTIGATIONAL PRODUCT

6.1 Identity of Investigational Product

The test product is volixibat potassium (formerly LUM002, formerly SHP626, hereafter referred to as volixibat) which will be provided in 5, 10 and 20 mg capsule form. Additional information is provided in the current Volixibat Potassium (SHP626) Investigator's Brochure.

The reference/comparator product is an identical PBO which will be provided in capsule form.

6.1.1 Blinding the Treatment Assignment

The IP will be supplied as double-blind blister packs. The actual double-blind treatment given to individual subjects is determined by a randomization schedule which will be automatically assigned by the interactive response technology (IRT). Placebo capsules, which exactly match the IP, will be used in the blister packs to provide the same number and size capsules for each of the doses within the treatment groups.

6.2 Administration of Investigational Product(s)

All IP and supplies will be provided by Shire or its designee**.** At each visit, subjects will be supplied with enough IP to last until the subsequent visit. Lost or damaged IP will be replaced as needed. Volixibat will be supplied to the clinical research center (CRC) as powder in capsule. Volixibat will be supplied in identical capsules in strengths of 5, 10, and 20 mg (with matched PBO). All doses of volixibat or matching PBO will be administered orally as a capsule in a double-blinded fashion. The first dose of IP for each subject will be administered in the clinic. The dose will be administered with 240 mL of water and should be given 30 minutes prior to the first meal of the day containing fat. All assessments should be completed at least 30 minutes prior to administration of the IP.

6.2.1 Interactive Response Technology for Investigational Product Management

IRT will be used for the following investigational tasks:

- Randomization
- Supply management
- Inventory management and supply ordering
- Expiration tracking

- Returns
- Emergency unblinding

6.2.2 Allocation of Subjects to Treatment

This is a double-blind, PBO-controlled study. The actual treatment given to individual subjects is determined by a randomization schedule.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation.

The randomization number represents a unique number corresponding to the IP allocated to the subject, once eligibility has been determined.

Individual subject treatment is automatically assigned by the IRT.

Once a randomization number/unique identifier has been assigned, that number must not be used again if, for example, a subject is withdrawn from the study. If a randomization number/unique identifier is allocated incorrectly, the clinical research associate (CRA)/study monitor must be notified as soon as the error is discovered.

IP packaging identification numbers, separate from randomization numbers/unique identifiers, may also be assigned to subjects for specific treatment assignment as dictated by the study. In these cases, the same IP packing identification number may not be assigned to more than 1 subject.

Subjects will be equally allocated to all volixibat doses and PBO whether their randomization precedes or follows the 24 week IA. The randomization will be stratified by treatment arm, baseline T2DM and NAS ≥ 6 or NAS = {4, 5}.

6.2.3 Dosing

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be broken during the study except in emergency situations where the identification of the IP is required for further treatment of the subject. The investigator should contact the CRO medical monitor and the sponsor medical monitor at the same time and as soon as possible after the investigator has been unblinded.

In the event that the treatment assignment is broken, the date, the signature of the person who broke the code and the reason for breaking the code are recorded on the IRT and the source

documents. Upon breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code-breaks that occur must be reported to the CRO medical monitor. Code-break information is held by the pharmacist/designated person at the site and by the CRO medical monitor for the study or designee.

For blinded studies, there will be a provision for unblinding to ensure adequate treatment of the subject in the case of an emergency.

6.3 Labeling, Packaging, Storage, and Handling

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled with a minimum of the protocol number, medication identification number, dosage form (including product name and quantity in pack), directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements 'For clinical trial use only', and/or 'CAUTION: New Drug - Limited by Federal (or USA) Law to Investigational Use', 'Keep out of reach of children', and the sponsor's name and address. Any additional labeling requirements for participating countries will also be included on the label.

Additional labels may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label
- Obscure the clinical study label
- Identify the study subject by name.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

The sponsor or designee will provide the IP for this study. The IP is packaged in the following labeled containers:

- Volixibat 5 mg capsules
- Volixibat 10 mg capsules
- Volixibat 20 mg capsules

- Volixibat PBO capsules

6.3.3 Storage

The investigator has overall responsibility for ensuring that the IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented.

IPs are distributed by the pharmacy or nominated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP labels as they are distributed.

All IP must be stored at the clinic at 20 - 25^{o}C (68 - 77^{o}F); excursions are allowed between 15 - 30^{o}C (59 - 86^{o}F).

IPs must be stored in accordance with labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the IP is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion below 15^{o}C (59^{o}F) or above 30^{o}C (86^{o}F); these excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the product be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room or a change in storage location.

6.4 Drug Accountability

Investigators will be provided with sufficient amounts of the IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for administering/dispensing the IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately

trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study following the procedures set out in the study protocol. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed IP will be documented on the eCRFs and/or other IP record. The investigator is responsible for assuring the retrieval of all study supplies from subjects.

No IP stock or returned inventory from a Shire-sponsored study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records.

With the written agreement of the sponsor, at the end of the study all unused stock, subject returned IP, and empty/used IP packaging may be destroyed at the site or a local facility. In this case, destruction records identifying what was destroyed, when and how, must be obtained with copies provided to the sponsor. Destruction of IP must be in accordance with local, state, and national laws.

Alternately, at the end of the study, or as instructed by the sponsor, all unused stock, subject-returned IP, and empty/used IP packaging are to be sent to a nominated contractor on behalf of the sponsor. IP being returned to the sponsor's designated contractors must be counted and verified by clinical site personnel and the sponsor (or designated CRO). For unused supplies where the original supplied tamper-evident feature is verified as intact, the tamper-evident feature must not be broken and the labeled amount is to be documented in lieu of counting. Shipment return forms, when used, must be signed prior to shipment from the site. Validated electronic return systems (ie, IRT) do not require a shipment form. Returned IP must be packed in a tamper-evident manner to ensure product integrity. Contact the sponsor for authorization to return any IP prior to shipment. Shipment of all returned IP must comply with local, state, and national laws.

6.5 Subject Compliance

Subjects must be instructed to bring their unused IP and empty/used IP packaging to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, blister pack) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details on the drug accountability form.

7. STUDY PROCEDURES

7.1 Study Schedule

This study will consist of a Screening period of up to 42 days, a 48-week Treatment period, and a Follow-up visit 4 weeks after treatment ends. A detailed display of all study procedures is provided in Table 1: Schedule of Assessments.

Patients will be assessed according to the following schedule:

- Screening – Visit 1 (Weeks -6 to 0 [Day -42 to Day -1])
- Baseline – Visit 2 (Week 0 [Day 0])
- Treatment and Assessments – Visits 3 through 9 (Week 1 through Week 48)
- Follow-up – Visit 10 (Week 52, 4 weeks after completion of dosing)

7.1.1 Screening (Visit 1)

Screening procedures must be completed within 42 days prior to randomization for the first dose of the IP. At the Screening visit, considered Visit 1 (Week -6 to 0, Day -42 to -1), all screening assessments and procedures are to be performed by the principal investigator or a qualified designee. See Table 1 for a complete list of screening procedures to be performed.

Written, signed, and dated informed consent from the subject prior to the performance of any study-related procedures must be obtained by the principal investigator or a designee. A copy of the signed informed consent form must be given to the subject for their records.

The following Screening procedures should be assessed at the beginning of the visit window, preferably between Day -42 and Day -35: inclusion/exclusion criteria, collection of subject information (demographics, medical and medication history AEs, and concomitant medications), a physical examination including vital signs, height, weight, and waist/hip measurements; collection of blood and urine samples for screening and safety assessments, and performance of an electrocardiogram (ECG). MRI and liver biopsy (if one has not been completed in the previous 6 months) should be performed with sufficient time to ensure results are received prior to the Baseline visit.

A screen failure is a subject who has given informed consent and failed to meet the inclusion and/or met at least 1 of the exclusion criteria and has not been randomized or administered the IP. Subjects cannot be rescreened once they have been designated as a screen failure.

7.1.2 Baseline (Visit 2)

Following the Screening visit, subjects will return to the clinic within 28 days for Visit 2, (considered Week 0, Day 0), for Baseline assessments including review of the inclusion/exclusion criteria, adverse events, and concomitant medications; physical examination with vital signs, weight, and waist/hip measurements; ECG; collection of blood and urine samples; and completion of the patient-reported outcome (PRO) EuroQol-5 Dimension-5 Level Questionnaire (EQ-5D-5L) survey. After completion of these assessments, subjects are randomized using Interactive Response Technology (IRT) to 1 of 4 treatment arms receiving volixibat 5 mg, 10 mg, or 20 mg, or PBO. The initial supply of study drug is dispensed to ensure adequate daily dosing until the next scheduled study visit.

7.1.3 Treatment and Assessment Period (Visits 3 through 9/EOS)

During the 48 weeks of double-blind treatment, 7 clinic visits are scheduled to occur as follows:

- Visit 3 - Week 2, Day 14 (+/- 3 days)
- Visit 4 - Week 4, Day 28 (+/- 3 days)
- Visit 5 - Week 8, Day 56 (+/- 5 days)
- Visit 6 - Week 12, Day 84 (+/- 5 days)
- Visit 7 - Week 24, Day 168 (+/- 7 days) – Interim Analysis
- Visit 8 - Week 36, Day 252 (+/- 7 days)
- Visit 9 - Week 48, Day 336 (+/- 7 days) – End of Study

Subjects are reminded not to eat and not to take their study drug on the day of scheduled study visits prior to completion of assessments. They should bring their study drug with them to the visit. All assessments should be completed at least 30 minutes prior to administration of the IP. IP should be taken 30 minutes prior to the first meal of the day containing fat.

The Schedule of Assessments provided in Table 1 details the procedures to be completed at each visit. All Treatment visits (3 through 9) will include weight and waist/hip measurements, assessment of vital signs, and blood sampling for completion of biochemistry, hematology, serum glucose, lipid panel, HbA1c, and serum liver-related blood tests. At all visits, adverse events, and concomitant medications will be collected for all subjects, and female subjects of childbearing potential will have a urine pregnancy test. Subjects will return containers of unused study drug for assessment of accountability and compliance which will be documented in the IRT. New supplies of study drug will be dispensed at Visits 3 through 8.

Additional assessments will also occur less frequently during the Treatment Period for coagulation panel, vitamin D, insulin, thyroid testing, urinalysis, ECG, physical examinations, and completion of the EQ-5D-5L.

An MRI will be repeated at Visit 7 for the 24-week IA and at Visit 9 (Week 48) for the subjects in the IA set only. A final liver biopsy will be performed at Visit 9 (Week 48) for all subjects unless a subject discontinues prior to Week 44, in which case the EOS liver biopsy is not required.

7.1.4 Follow-up (Visit 10)

The follow-up period for this protocol is 4 weeks after the last dose of study drug with a final Follow-up visit scheduled for Week 52. Procedures to be completed at this final visit include physical examination, weight, waist/hip measurements, vital signs, samples for biochemistry and hematology, serum glucose, lipid panel, HbA1c, serum liver-related blood tests, insulin, thyroid testing, and urine pregnancy test (for women of childbearing potential). Adverse events and concomitant medications will be recorded. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Section 8.1).

7.1.5 Additional Care of Subjects after the Study

No after care is planned for this study.

7.2 Study Evaluations and Procedures

All assessments and procedures are to be performed by the Investigator or a qualified designee who has been trained in the protocol. Assessments are to be performed according to the schedules shown in Table 1. If a subject terminates the study early, the CRC will make reasonable effort to perform the EOS and safety follow-up assessments and procedures for the subject's safety and well-being.

7.2.1 Demographic and Other Baseline Characteristics

Demographic details will be obtained at screening and recorded on the eCRF. Data collected will include age, gender, ethnicity, height, and weight.

7.2.2 Efficacy

7.2.2.1 Liver Biopsy

Liver biopsies will provide histologic data for confirmation of the diagnosis of NASH, assessment and grading of NASH activity, and scoring of steatosis, lobular inflammation, ballooning, as well as fibrosis and additional features (see Appendix 2 and Laboratory Manual for additional information).

7.2.2.2 MRI

The presence of ≥ 5% steatosis on screening MRI from a centrally read radiologist will be performed either during the Screening period or within 6 months prior to the first visit. Steatosis is assessed by MRI hepatic fat fraction. The 48-week MRI will be conducted for subjects in the IA set only.

7.2.3 Safety

7.2.3.1 Medical History and Medications

Medical history including important medical events and concomitant medication and illnesses will be obtained at Screening and will be recorded on the eCRF. Any existing medical condition present prior to the time of randomization should be reported as medical history

7.2.3.2 Physical Examination

A physical complete examination will be performed with a thorough review of body systems at Screening, Baseline prior to randomization, and at study visits specified in Table 1. Physical examinations will include a review of the subject's general appearance, as well as evaluation of the body systems including:

- Eyes, ears, nose, throat
- Lymph nodes
- Cardiovascular
- Skin
- Abdomen
- Neurological

- Spine and extremities

Abnormalities identified at the Screening visit (Visit 1) will be documented in the subject's source documents and on the medical history eCRF. Changes after the Screening visit (Visit 1) will be captured as AEs on the AE eCRF page, if deemed clinically significant by the investigator.

Height will be measured at the Screening visit only while weight and waist and hip circumference will be recorded at all study visits. BMI will be calculated programmatically.

7.2.3.3 Adverse Event Collection

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. (Please refer to Section 8, Adverse and Serious Adverse Events Assessment.)

7.2.3.4 Vital Signs

Vital signs include oral or tympanic temperature, sitting blood pressure, pulse, and respiratory rate. Blood pressure should be determined by cuff (using the same method, the same arm, and in the same position throughout the study). Any deviations from Baseline (Visit 2) vital signs which are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

7.2.3.5 Clinical Laboratory Evaluations

All clinical laboratory assays will be performed according to the central laboratory's normal procedures. Reference ranges supplied by the central laboratory will be used to assess the clinical laboratory data for clinical significance and out-of-range pathological changes. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant. Clinically significant findings should be evaluated for recording as adverse events on the eCRF. Abnormal clinical laboratory values, which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

The following clinical laboratory assessments will be performed:

Biochemistry

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Lactate dehydrogenase (LDH)
- Magnesium (Mg)

- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Blood urea nitrogen (BUN)
- C4
- Calcium (Ca)
- Bicarbonate (CO2)
- Chloride (Cl)
- Creatinine
- Creatine kinase
- Gamma glutamyl transferase (GGT)
- Glucose
- Phosphate (P)
- Potassium (K)
- Sodium (Na)
- Total and direct bilirubin
- Total cholesterol
- Protein
- Triiodothyronine (T3)
- Thyroid-stimulating hormone (TSH)
- Triglycerides
- Uric acid

Hematology

- Hemoglobin
- Hematocrit
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelets
- Red blood cell (RBC)
- White blood cell (WBC) count with differential
- Prothrombin time (PTT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Urinalysis

- Appearance (clarity and color)
- Bilirubin
- Blood
- Glucose
- Ketones
- Leukocyte esterase
- Microscopic examination of sediment
- Nitrite
- pH
- Protein
- Specific gravity
- Urobilinogen
- Oxalate

7.2.3.6 Additional Laboratory Assessments

Laboratory samples will be collected and assessed for:

- HIV testing and assessment of Hepatitis B/C including HBcAb, HBsAg, HBVDNA, and HCVAb, and HCVRNA will occur at the Screening visit only
- Lipid panel including fasting total cholesterol, HDL-C, LDL-C, and triglycerides at each scheduled study visit (Visits 1 through 10)
- HbA1c will be tested at every scheduled visit (Visits 1 through 10)
- Serum liver-related blood tests including ALT, AST, ALP, GGT, and TB will be analyzed at every visit (Visits 1 through 10)
- Full coagulation panel will be done at Screening and Baseline, but only INR and PT are required at remaining time points at Visits 4, 7, and 9
- Insulin will be tested at Screening, Baseline, at Visit 4 and Visits 6 through 10
- Vitamin D will be tested at Screening, Baseline, and Visits 4, 7, and 9
- Thyroid testing including TSH and T3 will be tested at Screening, Baseline, and Visits 4, 6, 7, 9, and 10
- C4 samples will be collected at Baseline and Visits 6, 7, 9, and 10

7.2.3.7 Pregnancy Test

A urine pregnancy test is performed on all females of child-bearing potential at the Screening visit (Visit 1), Baseline visit (Visit 2), at each Treatment visit (Visits 3 through 9) and at the Final visit (Visit 10), or if pregnancy is suspected, or on withdrawal of the subject from the study. A positive urine pregnancy test must be followed with a serum pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Section 8.1.7.

7.2.3.8 Drug and Alcohol Testing

A urine screen for drugs of abuse and blood test for alcohol will be performed at Screening and Baseline as described in Table 1. Additional drug and alcohol screens may be performed at the investigator's discretion.

Urine samples are to be tested for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiate metabolite, and phencyclidine.

Results of drug and alcohol screens will be reviewed and verified by the study monitor, but will not be collected in the eCRF database.

Any positive result for drugs of abuse at the Screening or Baseline visits will exclude the subject from further participation in the study. A positive test for drugs of abuse or alcohol done at the investigator's discretion discovered at any time during the study will be grounds for study discontinuation.

7.2.3.9 Electrocardiogram

An ECG (12-lead) will be performed at the times specified in Table 1 in accordance with the clinical site's standard practice(s) and equipment supplied by the CRC. The Baseline visit ECG will be completed in triplicate with a 10 minute interval between each reading. The remaining ECGs may consist of a single reading. Recordings of ECGs will be read locally at the clinical site by a qualified cardiologist. The ECG will include assessment of heart rate, sinus rhythm, atrial or ventricular hypertrophy, and assessment of PR, RR, QRS, and QT intervals. Identification of any clinically significant findings and/or conduction abnormalities will be recorded on the eCRF.

7.2.3.10 Health-related Quality of Life Assessments

The EuroQoL (Euroql. 2016. Available at: http://www.euroqol.org. [Accessed 17 February 16]) EQ-5D-5L ("EQ-5D") is a widely used standardized questionnaire that assesses generic HRQoL and is also recommended for health-economic evaluations. The EQ-5D includes two components: a descriptive profile and a visual analogue scale (VAS). The descriptive profile includes five dimensions (ie, pain/discomfort, mobility, usual activities, self-care and anxiety/depression), each with five levels (ie, no problems, slight problems, moderate problems, severe problems, extreme problems). An EQ-5D index can also be derived from the data which summarizes health status using a single value (ie, health-state utility). The psychometric properties of the EQ-5D-5L have been established and well documented (see Appendix 3).

7.2.3.11 Volume of Blood to be Drawn from Each Subject

During this study, it is expected that approximately 124 mL of blood will be drawn from all subjects, regardless of sex.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment; however, the total volume drawn over the course of the study should be approximately 124 mL. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

8. ADVERSE AND SERIOUS ADVERSE EVENTS ASSESSMENT

8.1 Definition of Adverse Events, Period of Observation, Recording of Adverse Events

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH Guidance E2A 1995).

All AEs are collected from the time the informed consent is signed until the defined follow-up period stated in Section 7.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not the IP is administered. Where possible, a diagnosis rather than a list of symptoms should be recorded. If a diagnosis has not been made, then each symptom should be listed individually. All AEs should be captured on the appropriate AE pages in the eCRF and in source documents. In addition to untoward AEs, unexpected benefits outside the IP indication should also be captured on the AE eCRF.

All AEs must be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to normal), regardless of whether the subject is still participating in the study. Closure indicates that an outcome is reached, stabilization achieved (the investigator does not expect any further improvement or worsening of the event), or the event is otherwise explained. When appropriate, medical tests and examinations are performed so that resolution of event(s) can be documented.

8.1.1 Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity should be captured as a new event. Worsening of pre-treatment events, after initiation of the IP, must be recorded as new AEs (for example, if a subject experiences mild intermittent dyspepsia prior to dosing of the IP, but the dyspepsia becomes severe and more frequent after first dose of the IP has been administered, a new AE of severe dyspepsia (with the appropriate date of onset) is recorded on the appropriate eCRF).

The medical assessment of severity is determined by using the following definitions:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Appendix 4 provides criteria to assess the severity of liver-related adverse events.

8.1.2 Relationship Categorization

A physician/investigator must make the assessment of relationship to the IP for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source document.

The following additional guidance may be helpful:

Term	Relationship Definition
Related	The temporal relationship between the event and the administration of the IP is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not Related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP and the event.

8.1.3 Outcome Categorization

The outcome of AEs must be recorded during the course of the study on the eCRF. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae

- Recovering/Resolving
- Unknown.

8.1.4 Symptoms of the Disease Under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected progression of the disease and are part of the efficacy data to be collected in the study; however, significant worsening of the symptoms should be recorded as an AE.

8.1.5 Clinical Laboratory and Other Safety Evaluations

A change in the value of a clinical laboratory, vital sign, or ECG assessment can represent an AE if the change is clinically relevant or if, during treatment with the IP, a shift of a parameter is observed from a normal value to an abnormal value, or a further worsening of an already abnormal value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing treatment or after the end of treatment with the IP, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign, or ECG values which were not present at the pre-treatment value observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease) is found for the abnormal values.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a clinical laboratory, vital sign, or ECG parameter is clinically significant and therefore represents an AE.

8.1.6 Criteria for Discontinuation of Treatment

Appendix 5 provides guidelines for discontinuation of treatment based on elevated ALT, AST, TB, and associated signs and symptoms.

8.1.7 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until the defined follow-up period stated in Section 7.1.4.

Any report of pregnancy for any female study participant (or the female partner of a male participant) must be reported within 24 hours to the Shire Global Pharmacovigilance and Risk Management Department using the Shire Investigational and Marketed Products Pregnancy Report Form. A copy of the Shire Investigational and Marketed Products Pregnancy Report Form (and any applicable follow-up reports) must also be sent to the CRO/Shire Medical Monitor using the details specified in the emergency contact information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days post partum.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form. Note: An elective abortion is not considered an SAE.

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form as well as the Shire Investigational and Marketed Products Pregnancy Report Form. The test date of the first positive serum/urine β-HCG test or ultrasound result will determine the pregnancy onset date.

8.1.8 Abuse, Misuse, Overdose, and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE as described in Section 8.2. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of the IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society

- Misuse – Intentional use of the IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol)

- Overdose – Intentional or unintentional intake of a dose of an IP exceeding a pre-specified total daily dose of the product.

- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

8.2 Serious Adverse Event Procedures

8.2.1 Reference Safety Information

The reference for safety information for this study is the Volixibat Potassium (SHP626) Investigator's Brochure which the sponsor has provided under separate cover to all investigators.

8.2.2 Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Shire Global Pharmacovigilance and Risk Management Department and the CRO/Shire Medical Monitor within 24 hours of the first awareness of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Section 8.1.8) unless they result in an SAE.

The investigator must complete, sign, and date the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form and verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested) and fax or e-mail the form to the Shire Global Pharmacovigilance and Risk Management Department. A copy of the Shire Clinical Study Serious Adverse Event and Non-serious AEs Required by the Protocol Form (and any applicable follow-up reports) must also be sent to the CRO/Shire Medical Monitor using the details specified in the emergency contact information section of the protocol.

8.2.3 Serious Adverse Event Definition

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence (whether considered to be related to the IP or not) that at any dose:

- Results in death
- Is life-threatening. Note: The term 'life-threatening' in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization. Note: Hospitalizations, which are the result of elective or previously scheduled surgery for pre-existing conditions, which have not worsened after initiation of treatment, should not be classified as SAEs. For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Is a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. 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.

All SAEs (regardless of relationship to study) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 7.1.4, and must be reported to the Shire Global Pharmacovigilance and Risk Management Department and the CRO/Shire Medical Monitor within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Shire Global Pharmacovigilance and Risk Management Department within 24 hours of the first awareness of the event.

8.2.4 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms experienced by the subject after signing the informed consent form, or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

8.2.5 Fatal Outcome

Any SAE that results in the subject's death (ie, the SAE was noted as the primary cause of death) must have fatal checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received the IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

8.2.6 Regulatory Agency, Institutional Review Board, Ethics Committee, and Site Reporting

The CRO is responsible for notifying the relevant regulatory authorities as appropriate: USA central IRBs/EU central ECs of related, unexpected SAEs.

In addition the CRO is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the SHP626-201 program.

The investigator is responsible for notifying the local IRB, local EC, or the relevant local regulatory authority of all SAEs that occur at his or her site as required.

9. DATA MANAGEMENT AND STATISTICAL METHODS

9.1 Data Collection

The investigators' authorized site personnel must enter the information required by the protocol on the eCRF. A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting. Once a subject is randomized, it is expected that site personnel will complete the eCRF entry within approximately 3 business days of the subject's visit.

9.2 Clinical Data Management

Data are to be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

9.3 Data Handling Considerations

Data that may potentially unblind the treatment assignment (ie, IP serum concentrations, antibiotics to investigation product, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

9.4 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for

summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused and spurious data will be addressed.

The SAP will be finalized prior to unblinding to preserve the integrity of the statistical analysis and study conclusions.

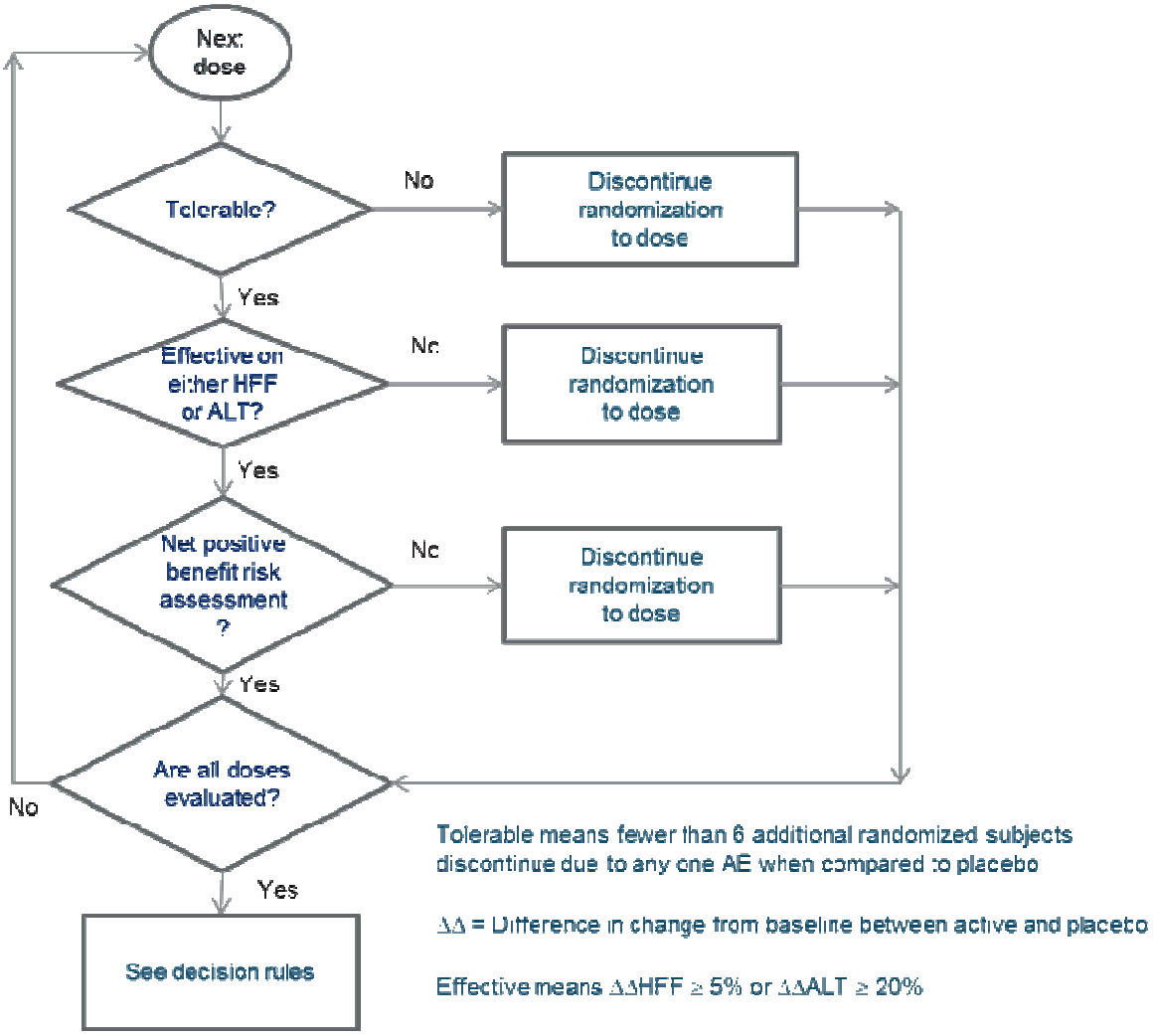
All statistical analyses will be performed using SAS® (SAS Institute, Cary, NC 27513).

9.5 Planned Interim Analysis

Study enrollment will be paused after 92 subjects have been randomized in order for an IA to be conducted by an independent DMC after at least 80 subjects have received 24 weeks of treatment. The IA will evaluate the safety, tolerability and efficacy of 3 doses of volixibat each compared to PBO. The Interim Analysis Set (IAS; see Section 9.7) will be used for the analyses conducted at the IA. The IA will be used to drop one or more doses of volixibat, or to terminate the trial.

Decisions about whether or not to continue randomizing to a particular dose following the 24 week IA should be made in accord with Figure 2 and its associated decision rules. In the figure, a net positive benefit risk assessment implies that the drug is more effective than any negative safety concerns. Effectively, randomization should be discontinued to any dose determined to be intolerable or to have a net negative benefit risk. Hepatic fat fraction as measured by MRI is abbreviated HFF in the figure. The liver enzyme alanine aminotransferase is abbreviated ALT.

Figure 2: Process Flow for Volixibat Dose Decisions at 24 Week IA



Decision Rules:

If randomization is discontinued to at least one dose, then randomize equally to the remaining volixibat dose(s) and PBO. That is, if two doses are determined to be tolerable and to have a net positive benefit risk assessment, then randomization to those two doses (and PBO) should continue. Further, if only one dose is determined to be tolerable and to have a net positive benefit risk assessment, then randomization to only that dose (and PBO) should continue.

Otherwise, the choice of doses to which randomization should continue should be made according to the following general principles and specific rules which are embodied in Appendix 6.

Subjects recruited into the study prior to the IA will continue with the same dosing regimen for the duration of the planned study participation. That is, subjects receiving a dose to which randomization is stopped at the IA will continue to receive that same dose throughout the course of the study, and complete all required assessments. Subjects recruited in the study after completion of the IA will be randomized evenly to the remaining dose groups or PBO group by IRT. Subjects will be randomized to at most two volixibat doses as well as to PBO. Thus, depending on the results from the IA, the study may be terminated, or one or more doses of volixibat will be discontinued. If the study is not terminated, subjects will receive a total of 48 weeks of IP.

9.5.1 Tolerability

Tolerability will be assessed by the number of discontinuations due to any one TEAE. The most common causes of discontinuation seen in healthy human trials were gastrointestinal events, most notably, diarrhea, loose stools, increased evacuations, and abdominal pain. Thus, calculations used to determine dropping one or more doses of volixibat in this study were based upon the rate of evacuations seen in the Phase 1 trial SHP626-101, as this TEAE was felt to be most objectively measured. The average rate of evacuations was ~ 1.27 per subject per day for PBO. The lowest rate of evacuations on volixibat occurred on 10 mg, and was ~ 1.64 per subject per day. If this frequency of evacuations translates to average numbers of discontinuations, a difference of 6 or more discontinuations on volixibat when compared to PBO would occur by chance with less than 1% probability. Consequently, if 6 or more additional subjects treated with a specific dose of volixibat discontinue due to any particular TEAE when compared to PBO (absolute difference) then randomization to that dose will be stopped. A particular volixibat dose will be considered tolerable if fewer than 6 additional subjects discontinue due to any TEAE when compared to PBO. If none of the doses are tolerated, the study will be terminated.

9.5.2 Efficacy

Efficacy at the IA will be based on reduction of steatosis (as measured change from Baseline in HFF on MRI). If a volixibat dose does not achieve efficacy as measured by HFF reduction on MRI, change from Baseline in ALT at Week 24 will then be evaluated.

The sample size of 80 subjects provides 80% power to test for a net difference in HFF (volixibat dose minus PBO) of 5% at the two-sided 10% significance level. The net difference of 5% was based on the colesevelam clinical trial (Davidson et al. 2010). The statistical method used to test reduction in HFF will be an ANCOVA model which includes change from Baseline as the dependent variable, and treatment and baseline HFF as independent variables. Treatment will be a class variable and baseline HFF will be continuous. Each volixibat dose will be separately compared to PBO to assess whether its net reduction is significantly different from zero at the 10% significance level. A Holm multiplicity adjustment will be applied. If the dose is significantly different from PBO, then that dose will be considered effective at the interim assessment.

If a volixibat dose does not achieve efficacy as measured by HFF reduction on MRI, percent change from Baseline in ALT at 24 weeks will be evaluated. If the difference is at least 20% when compared to PBO, then that dose will be considered effective at the interim assessment. In addition, if multiple doses exhibit a difference of a least 20% when compared to PBO then the statistical method used to test reduction in ALT will be an ANCOVA model which includes change from Baseline as the dependent variable, and treatment and baseline ALT as independent variables. Treatment will be a class variable and baseline ALT will be continuous. Each volixibat dose will be separately compared to PBO to assess whether its net reduction is significantly different from zero at the 10% significance level. A Holm multiplicity adjustment will be applied. If the dose is significantly different from PBO, then that dose will be considered effective at the interim assessment.

Data Monitoring Committee

An independent DMC will be established to assess safety, tolerability and efficacy during the study, as well as to ensure the validity and scientific merit of the trial. In addition, the DMC will evaluate efficacy of different doses of volixibat at the 24 week IA (using the process flow and decision rules specified previously). Based on their evaluation, the DMC will make recommendations concerning study discontinuation due to intolerability or futility, or the continuation of one or more dose groups to the 48 week liver biopsy. The DMC will monitor ongoing data generated by the study at regular intervals for the duration of the study. Their role is to protect the interests of the subjects in the study and of those still to be entered, by review of accumulating data generated in the study.

The roles, responsibilities and rules governing operation of the DMC will be discussed in full in a DMC charter. The DMC charter will define the primary responsibilities of the DMC; guide its activities, its relationship with other study components, its membership, and the purpose and timings of its meetings. It will provide the procedures for ensuring confidentiality, formal communication, and outline of the content of reports that will be provided by the DMC. Data provided to the DMC will not be considered ‘clean’ until the database is locked.

Appropriate summary statistics and data listings will be provided to the DMC by an independent statistician supported by an independent statistical reporting group not otherwise assigned to the study.

The recommendations made by the DMC to alter the conduct of the study will be forwarded to Shire for final decision. Shire will forward such decisions to regulatory authorities, as appropriate.

9.6 Sample Size Calculation and Power Considerations

Primary hypothesis: The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from Baseline NAS differs between at least one volixibat dose and PBO.

Response rates of 21% (PBO) and 45% (active) were reported in the FLINT trial (Neuschwander-Tetri et al. 2015) using the same primary end-point of reduction of NAS by at least two points (without worsening of fibrosis). Based on those response rates, 67 subjects per group completing the trial are needed for 80% power with a 10% type I error for a study comparing two volixibat doses to PBO. Holm multiplicity adjustment was included in the sample size estimate.

Approximately 334 subjects will be screened to enroll 266 subjects to achieve 201 completers. After enrollment of at least 80 subjects (20/treatment arm – 3 active and 1 PBO) a 24-week IA will eliminate at least one dose group based on the criteria provided to the DMC; enrollment will continue to include approximately 67 subjects in each of the remaining arms. The sample size target for this study is 67 completers in each of the treatment groups (201 total if 3 arms continue). Including an additional 20 subjects to account for one dose dropped at the IA, 221 subjects are needed. Therefore, approximately 266 (accounts for 20% of 221 dropping out) subjects will be randomized if three arms (2 SHP626 and 1 PBO) continue. Fewer subjects are needed if more than one SHP626 dose is dropped.

9.7 Study Population

The Screened Set will consist of all subjects who have signed an informed consent.

The Safety Analysis Set will consist of all subjects who take at least 1 dose of randomized IP, and have at least 1 post-Baseline safety assessment (eg, coming back for any visit, reporting of an AE or reporting the absence of AEs).

The Full Analysis Set (FAS) will consist of all subjects in the Safety Analysis Set who have a valid scored liver biopsy.

The IAS will consist of all subjects in the Safety Set who have a valid post-baseline hepatic fat fraction assessed by MRI and a valid post-baseline ALT at 24 weeks.

9.8 Efficacy Analyses

All efficacy analyses will be based on the FAS and all statistical tests will be 2-sided hypothesis tests performed at the 10% level of significance. Also, all confidence intervals will be 2-sided confidence intervals, unless otherwise stated.

9.8.1 Primary Efficacy Endpoint

The primary endpoint is the binary response indicating (yes/no) whether a subject responded at Week 48 with a reduction, of at least 2 points without worsening of fibrosis, from baseline NAS.

The FAS will be used to assess the primary efficacy endpoint. The proportion of subjects at Week 48 with a reduction of at least 2 points without worsening of fibrosis from baseline NAS will be analyzed. The difference between a volixibat dose and PBO will be tested with a stratified Cochran-Mantel-Haenzel test. The test will be stratified by presence or absence of T2DM at baseline and baseline NAS separated into two groups (NAS={4, 5} or NAS={6,7,8}). Holm multiplicity adjustment will be applied prior to determining statistical significance at the 0.1 level.

Sensitivity analyses may also be conducted in addition to the primary analysis. If the proportion of subjects missing the Week 48 liver biopsy reading does not exceed 10%, then no sensitivity analyses will be conducted for the primary efficacy endpoint. Otherwise, analyses which explore the impact of the missing data on the primary efficacy endpoint will be conducted. These sensitivity analyses may compare imputations of the missing values which favor the PBO and/or imputations which favor the active. Further, if the pattern of missing values does not appear uniformly distributed among the treatment arms, imputation method(s) based on informative missingness may also be performed. The sensitivity analyses will be detailed in the Statistical Analysis Plan.

9.8.2 Secondary Efficacy Endpoints

- Change from Baseline to Week 48 in safety and tolerability of volixibat as measured by the number of subjects discontinuing treatment due to any one treatment-emergent adverse event (TEAE).

- Change from Baseline to Week 48 on liver histology as measured by the individual NAS components (ballooning, inflammation, steatosis).

- Change from Baseline to Week 48 on hepatic steatosis as measured by MRI-HFF for subjects included in the IA.

- Change from Baseline to Week 48 on liver histology as measured by fibrosis stage. (NASH CRN system and Ishak)

- Resolution of NASH (defined as an overall histologic interpretation of no fatty liver disease or simple steatosis without steatohepatitis or isolated steatosis without steatohepatitis) without worsening of fibrosis as assessed by liver histology at Week 48.

- Change from Baseline to Week 48 on serum liver-related biochemistry as measured by:

    - alanine aminotransferase (ALT)

    - aspartate aminotransferase (AST)

    - alkaline phosphatase (ALP)

    - gamma glutamyl transferase (GGT)

  - total bilirubin (TB)

- Change from Baseline to Week 48 on metabolic indicators as measured by:
  - fasting serum glucose levels
  - insulin levels
  - HbA1c
- Change from Baseline to Week 48 on serum lipids measured by:
  - fasting total cholesterol
  - HDL-C
  - LDL-C
  - triglycerides

Descriptive statistics for all secondary endpoints will be presented for each time point at which the variable is measured (see Table 1: Schedule of Assessments).

9.8.3 Exploratory Efficacy Endpoints

- Change from Baseline to Week 48 on liver histology as measured by the SAF scoring components: Steatosis (S), Activity (A), and Fibrosis (F)
- Change from Baseline to Week 48 on anthropomorphic measures:
  - body weight
  - BMI
  - waist circumference
  - waist-hip ratio
- Change from Baseline to Week 48 in subjects with T2DM on homeostasis measured by:
  - Homeostasis model assessment 2-IR (HOMA2-IR)
  - HOMA2-beta cell function (HOMA2-%B)
- Change from Baseline to Week 48 on the EQ-5D index, a patient-reported-measure of HRQL and health status.

- Change from Baseline to Week 48 on the EQ-5D VAS score.
- Change from Baseline to Week 48 in the proportion of subjects reporting having problems (no problems, slight problems, moderate problems, severe problems, extreme problems) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire

Descriptive statistics for all exploratory endpoints will be presented for each time point at which the variable is measured (see Table 1: Schedule of Assessments).

9.9 Safety Analyses

The Safety Set will be used to assess the safety endpoints.

The number of discontinuations due to any 1 TEAE will be summarized for each treatment group.

The number of events, incidence, and percentage of TEAEs will be presented for each treatment group by system organ class, and by the preferred term using the agreed upon version of the Medical Dictionary for Regulatory Activities.

Treatment-emergent AEs will be further summarized by severity.

Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will also be summarized.

Vital signs, ECG findings, and clinical laboratory tests will be summarized for each treatment group. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary

Adverse events will be coded using the Medical Dictionary for Regulatory Activities. The number of events, incidence, and percentage of TEAEs will be calculated overall, by SOC, by preferred term, and by treatment group. Treatment-emergent adverse events will be further summarized by severity and relationship to the IP. Adverse events related to the IP, AEs leading to withdrawal, SAEs, and deaths will be similarly summarized/listed.

Vital signs, ECG findings, and clinical laboratory tests will be summarized for each treatment group. Potentially clinically important findings will be summarized. Graphical presentation may be used when deemed necessary.

9.10 Other Analyses

Serum samples to be stored.

9.10.1 Health-related Quality of Life Analyses

- Change from Baseline to Week 48 on the EQ-5D index (Appendix 3). The EQ-5D index will be derived using published UK-based preference weights. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from Baseline to Week 48 on the EQ-5D VAS score. Univariate descriptive statistics and multivariate regression analyses adjusting for selected baseline covariates will be undertaken.

- Change from Baseline to Week 48 in the proportion of subjects reporting having problems (none, slight, moderate, severe, extreme) with pain/discomfort, mobility, usual-activities, self-care, anxiety/depression in the EQ-5D questionnaire. Proportions and 95% confidence intervals will also be generated for the baseline and Week 48 values as well as for change from baseline.

9.10.2 Stool Assessment

Stool hardness and number of evacuations will be assessed throughout the study. Stool hardness will be assessed for the softest evacuation within 24 hours of each clinic visit using the Bristol Stool Chart, a medical aid designed to classify the form of human feces into 7 categories where Type 1 is the hardest and Type 7 is the softest. Appendix 7 provides a sample of the Bristol Stool Chart. Number of evacuations within the past 24 hours prior to the clinic visit will be recorded at specified times as per Table 1.

10. SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study is conducted in accordance with current applicable regulations, ICH, EU Directive 2001/20/EC and its updates, and local ethical and legal requirements.

The name and address of each third party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

10.1 Sponsor's Responsibilities

10.1.1 Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of the IP for shipment to the site.

10.1.2 Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO as necessary.

10.1.3 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

10.1.4 Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of the end of the study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance. The sponsor will provide the ECs with a copy of the same summary.

10.1.5 Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and IRBs/ECs are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

The sponsor will make an end-of-study declaration to the relevant competent authority as required by Article 10 (c) of Directive 2001/20/EC.do

10.2 Investigator's Responsibilities

10.2.1 Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6 (1996), EU Directive 2001/20/EC, and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such

qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

10.2.2 Protocol Adherence and Investigator Agreement

The investigator and any co-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all the IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

10.2.3 Documentation and Retention of Records

10.2.3.1 Electronic Case Report Forms

eCRFs are supplied by the sponsor and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. eCRFs must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

All data sent to the sponsor must be endorsed by the investigator.

The CRA/study monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

Incorrect entries must be crossed with a single line as to not obscure the original entry. Corrections must be made adjacent to the item to be altered, initialed, and dated by an authorized investigator or designee as stated in the site delegation log. Overwriting of this information or use of liquid correcting fluid is not allowed.

All data sent to the sponsor must be endorsed by the investigator.

Once the CRA/study monitor has verified the contents of the completed eCRF pages against the source data, the duplicate pages are retrieved and forwarded to the study sponsor (or designee) for data entry. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

10.2.3.2 Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to: subject's medical file, subject diary cards, original clinical laboratory reports, and histology and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/EC, and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, images, etc.). Non-study site personnel will not disclose any personal information or personal medical information.

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, EMA, UK Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

10.2.3.3 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other USA national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

10.2.3.4 Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in the IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

10.3 Ethical Considerations

10.3.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from all study subjects prior to any study-related procedures including screening assessments. All consent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally-authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally-authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The principal investigator provides the sponsor with a copy of the consent form which was reviewed by the IRB/EC and which received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor, prior to the start of the study unless it is agreed to and documented (abiding by

regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

10.3.2 Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

For sites within the EU, the applicant for an EC opinion can be the sponsor, the investigator, or for multicenter studies the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue.

IP supplies will not be released until the Sponsor or its designee has received written IRB/EC approval of and copies of revised documents.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol, but in any case at least once a year; for sites within the EU, this can be done by the sponsor, the investigator or for multicenter studies the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs.

10.4 Privacy and Confidentiality

All USA-based sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the Sponsor or its designee.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the

data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market volixibat; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies – containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth – will be recorded. They may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

10.5 Study Results / Publication Policy

Shire will endeavor to publish the results of all qualifying, applicable, and covered studies according to external guidelines in a timely manner regardless of whether the outcomes are perceived as positive, neutral, or negative. Additionally, Shire adheres to external guidelines (eg, Good Publication Practices 2) when forming a publication steering committee, which is done for large, multicenter Phase 2-4 and certain other studies as determined by Shire. The purpose of the publication steering committee is to act as a non-commercial body that advises or decides on dissemination of scientific study data in accordance with the scope of this policy.

All publications relating to Shire products or projects must undergo appropriate technical and intellectual property review, with Shire agreement to publish prior to release of information. The review is aimed at protecting the sponsor's proprietary information existing either at the commencement of the study or generated during the study. To the extent permitted by the publisher and copyright law, the principal investigator will own (or share with other authors) the copyright on his/her publications. To the extent that the principal investigator has such sole, joint or shared rights, the principal investigator grants the sponsor a perpetual, irrevocable, royalty-free license to make and distribute copies of such publications.

The term "publication" refers to any public disclosure including original research articles, review articles, oral presentations, abstracts and posters at medical congresses, journal supplements, letters to the editor, invited lectures, opinion pieces, book chapters, electronic postings on medical/scientific websites, or other disclosure of the study results, in printed, electronic, oral or other form.

Subject to the terms of the paragraph below, the investigator shall have the right to publish the study results, and any background information provided by the sponsor that is necessary to

include in any publication of study results, or necessary for other scholars to verify such study results. Notwithstanding the foregoing, no publication that incorporates the sponsor's confidential information shall be submitted for publication without the sponsor's prior written agreement to publish, and shall be given to the sponsor for review at least 60 days prior to submission for publication. If requested in writing by Shire, the institution and principal investigator shall withhold submission of such publication for up to an additional 60 days to allow for filing of a patent application.

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results. If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single-site data being presented.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors (ICMJE) current standards. Participation as an investigator does not confer any rights to authorship of publications.

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12. APPENDICES

APPENDIX 1 PROTOCOL HISTORY

Document	Date	Global/Country/Site Specific
Original Protocol	24 February 2016	Global

APPENDIX 2 FATTY LIVER DISEASE HISTOLOGY SCORING

Diagnosis: NASH, Suspicious/Borderline NASH (type a or b), NAFLD, Not NAFLD

NAS SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Lobular Inflammation	0	no foci
		1	< 2 foci per 200x field
		2	2-4 foci per 200x field
		3	> 4 foci per 200 x field
	Ballooning	0	None
		1	Rare or diagnostically borderline
		2	Many or Prominent ballooned hepatocytes

SAF SCORING	Steatosis	0	<5%
		1	5-33%
		2	34-66%
		3	67-100%
	Ballooning	0	normal hepatocytes with cuboidal shape and pink cytoplasm
		1	clusters of hepatocytes with rounded shape and pale cytoplasm usually reticulated
		2	grade 1 plus enlarged hepatocytes, 2 x bigger than normal cells.
	Lobular Inflammation	0	none
		1	< 2 foci per 200x field
		2	>2 foci per 200x field

Steatosis 1,2,3 + Ballooning 1,2 + Lobular Inflammation 1,2 = NASH
Steatosis 1,2,3 +Ballooning 0 + Lobular Inflammation 0,1,2 = NAFLD
Steatosis 1,2,3 + Ballooning 1,2 + Lobular 0 = NAFLD
Steatosis 0 = no NAFLD

FIBROSIS SCORE	None	0
	Mild zone 3 perisinusoidal (requires trichrome)	1a
	Moderate Zone 3 perisinusoidal (visible on H&E)	1b
	Portal/periportal only	1c
	Portal, periportal and perisinusoidal	2
	Bridging	3
	Cirrhosis	4

References: Kleiner et al. 2005; Bedossa 2012

ADDITIONAL SCORING FEATURES

Expanded Balloon Score

0: None

1: Few Non classic

2: Few Classic

3. Many Classic

4: Severe Classic

Portal Inflammation

0: None

1: Minimal

2: Mild

3: More than Mild

Megamitochondria

0: None

1: Present

Acidophil Bodies

0: None or rare

1: Present

For stage 2 or greater fibrosis

Portal Predominant

Central Predominant

No predominance

Steatosis Zone

Zone 1 predominant

Zone 3 predominant

Azonal

Panacinar

Glycogenosis

None

Focal

Diffuse

APPENDIX 3 EQ-5D-5L HEALTH QUESTIONNAIRE UK SAMPLE ONLY, NOT FOR OFFICIAL USE

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

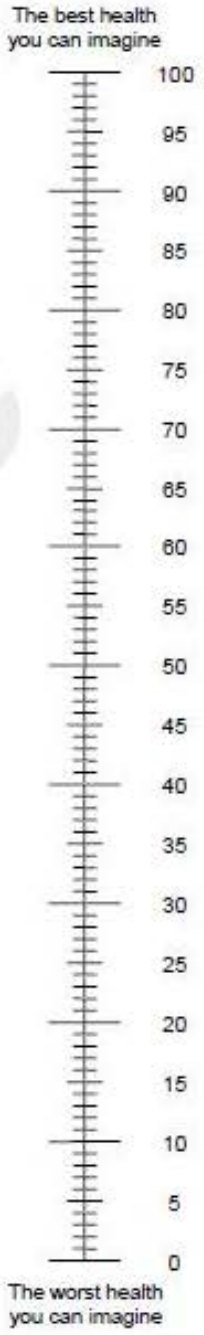
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX 4 CRITERIA TO ASSESS SEVERITY OF LIVER-RELATED ADVERSE EVENTS

Parameter	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
ALT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
AST	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Alkaline Phosphatase	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
GGT	<1.25	1.25-2.5	>2.5-5.0	>5.0-10	>10
Bilirubin	Normal	>1.0-1.5	>1.5-2.5	>2.5-5	>5

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma glutamyl transferase.
NOTE: Values expressed as multiples of the upper limit of the normal range (ULN).

APPENDIX 5 STOPPING RULES FOR LIVER-RELATED BLOOD TESTS

- ALT or AST >6xULN (confirmed with repeat within 24hrs)
- ALT or AST >5xULN for more than 2 weeks
- ALT or AST >3xULN **and** (TBL >2xULN **or** INR >1.5)
- ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

If ALT or AST elevations do not fall within the above parameters, but are > 4 ×ULN subject should remain on study drug with close observation which includes:

1. Immediately contacting the Shire medical monitor.
2. Repeating liver enzyme and serum bilirubin tests two or three times weekly.

   Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
3. Obtaining a more detailed history of symptoms and prior or concurrent diseases.
4. Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
5. Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease., or other disease at the discretion of the investigator
6. Obtaining a history of exposure to environmental chemical agents.
7. Obtaining additional tests to evaluate liver function, as appropriate (eg, INR, direct bilirubin).

APPENDIX 6 GENERAL PRINCIPLES AND SPECIFIC RULES FOR DOSE DISCONTINUATION AT 24 WEEK INTERIM ANALYSIS – GUIDANCE FOR DATA MONITORING COMMITTEE ONLY

General Principles

A net positive benefit risk assessment on HFF takes precedence over effectiveness on ALT. Covers the situations when 2 doses show net positive benefit risk on HFF and 1 dose shows positive or net positive benefit risk on ALT.

Choose the dose(s) that have the largest difference in dose from amongst those exhibiting a net positive benefit risk.

As a minimally absorbed drug, volixibat at higher doses is less likely than absorbed drugs to exhibit additional TEAEs in large longer duration trials that were not seen in smaller shorter trials.

Specific Rules

If 3 doses are determined to be tolerable and to have a net positive benefit risk assessment on HFF, then Scheffe's statistical multiple comparisons test should be applied to the three ΔΔHFF values. The test will result in one of eighteen possibilities. The 18 possible cases are detailed below in Table 1. For example, if the multiple comparisons test indicates that the low dose (L) is less effective on HFF than the medium and high doses (M and H) then the medium and high doses should be selected for continued randomization from among the three doses. This case is depicted as case 1 in the table. If doses are indistinguishable on ΔΔHFF then use Scheffe's test on ΔΔALT (case 2).

Table 1 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in Hepatic Fat Fraction

Case	Results	Action
1	L < M ≅ H	M, H
2	L ≅ M ≅ H	If can't decide using ALT, then L,H
3	L > M ≅ H	L, If can't decide using ALT, then L,H
4	L ≅ M > H	L, M
5	L > M > H	L, M
6	L < M < H	M, H

Table 1 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in Hepatic Fat Fraction

Case	Results	Action
7	L ≅ M < H	H, If can't decide using ALT, then L,H
8	M < L ≅ H	L, H
9	M > L ≅ H	M, If can't decide using ALT, then H
10	M ≅ L > H	L, M
11	M > L > H	L, M
12	M < L < H	L, H
13	M ≅ L < H	H, If can't decide using ALT, then M
14	L < H ≅ M	M, H
15	L > H ≅ M	L, If can't decide using ALT, then H
16	L ≅ H > M	L, H
17	L > H > M	L, H
18	L < H < M	M, H

L = low dose (5 mg), M = medium dose (10 mg), H = high dose (20 mg)

If 3 doses are determined to be tolerable and to have a net positive benefit risk assessment on only ALT, then Scheffe's statistical multiple comparison test should be applied to the three ΔΔALT values. The test will result in one of eighteen possibilities. The 18 possible cases are detailed below in Table 2. For example, if the multiple comparison test indicates that the low dose (L), medium dose (M) and high dose (H) are indistinguishably effective on ALT, then the low and high dose should be selected. This case is depicted as case 2 in the table.

Table 2 Possible Results from Applying a Multiple Comparison Test to Three Doses based on Change from Baseline in ALT

Case	Results	Action
1	L < M ≅ H	M, H
2	L ≅ M ≅ H	L,H
3	L > M ≅ H	L and H
4	L ≅ M > H	L, M
5	L > M > H	L, M
6	L < M < H	M, H
7	L ≅ M < H	H, L
8	M < L ≅ H	L, H
9	M > L ≅ H	M, H
10	M ≅ L > H	L, M
11	M > L > H	L, M
12	M < L < H	L, H
13	M ≅ L < H	H, L
14	L < H ≅ M	M, H
15	L > H ≅ M	L, H
16	L ≅ H > M	L, H
17	L > H > M	L, H
18	L < H < M	M, H

L = low dose (5 mg), M = medium dose (10 mg), H = high dose (20 mg)

If three doses are tolerable and effective and only one of them is tolerable and exhibits effectiveness on HFF, then randomization to that dose should continue. One of the remaining doses which are tolerable and effective only on ALT should be chosen based on a Scheffe's multiple comparisons test for ΔΔALT. If neither of the remaining two separates from the other with respect to ΔΔALT, then randomization should continue to the highest dose of the remaining two active doses (and PBO).

APPENDIX 7 BRISTOL STOOL CHART