TITLE PAGE

Protocol Title: A two-period study in healthy male participants to determine the pharmacokinetics, balance/excretion, and metabolism of [^{14}C]-linerixibat following a single intravenous radiolabeled microtracer dose (concomitant with a non-radiolabeled oral dose) and a single oral radiolabeled dose

Protocol Number: 205895

Compound Number: GSK2330672

Study Phase: Phase 1

Short Title: Pharmacokinetics and metabolism of radiolabelled linerixibat

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Regulatory Agency Identifying Number(s):

IND: 130391

EudraCT: 2019-000875-18

Approval Date: 11-APR-2019

SPONSOR SIGNATORY:

PPD

4/11/2019

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1. PROTOCOL SUMMARY

1.1. Synopsis

Protocol Title: A two-period study in healthy male participants to determine the pharmacokinetics, balance/excretion, and metabolism of [^{14}C]-linerixibat following a single intravenous radiolabeled microtracer dose (concomitantly with a non-radiolabelled oral dose) and a single oral radiolabeled dose

Short Title: Pharmacokinetics and metabolism of radiolabelled linerixibat

Rationale:

Absorption, metabolism and excretion of linerixibat (GSK2330672) have been studied in pre-clinical animal models, in vitro, and in previous clinical trials. However, no dedicated clinical studies of drug absorption, metabolism, and excretion have been conducted for linerixibat. Using radiolabelled linerixibat with Accelerator Mass Spectrometry (AMS) will enable quantitative measurement of linerixibat concentrations and comprehensive identification and quantification of drug metabolites that would not otherwise be possible.

This open-label study in 6 healthy male participants will assess the pharmacokinetics, balance/excretion, and metabolism of linerixibat in humans using [^{14}C]-radiolabelled drug substance administered as an intravenous (IV) infusion and orally. [^{14}C]-linerixibat administered by IV infusion will be a microtracer dose; therefore, it will be administered concomitantly with an oral non-radiolabelled dose, to ensure that the pharmacokinetics (PK) are representative of a clinically-relevant dose. Use of a string bile collection device for sampling duodenal bile after IV [^{14}C]-linerixibat infusion will enable a qualitative assessment of drug metabolites in this matrix to characterise biliary elimination pathways (Guiney, 2011). The study will also provide an assessment of linerixibat metabolism following administration of a [^{14}C]-radiolabelled oral solution.

Objectives and Endpoints:

Objectives	Endpoints[1]
Primary	
• To determine parent linerixibat and total drug-related radioactivity systemic concentrations following a single IV microtracer dose of [^{14}C]-linerixibat (concomitant with an oral dose of non-radiolabelled linerixibat) and oral dose of [^{14}C]-linerixibat[1]	• AUC(0–inf), AUC(0–t), C_{max}, t_{max} and $t_{1/2}$ of parent and total drug-related material (radioactivity) in plasma. • Volume (Vss) and clearance (CL) of parent after IV dose (Period 1 only). • Renal clearance of parent (CLr) after both IV and oral dose
• To estimate the oral bioavailability of linerixibat	• Direct estimation of F (absolute oral bioavailability), indirect calculation of Fh (fraction of drug escaping first pass hepatic clearance), and Fa (fraction absorbed)
• To determine the rate and extent of excretion of total radioactivity in urine and feces and the total recovery of radioactivity, following IV and oral administrations of [^{14}C]-linerixibat	• Urinary and fecal cumulative excretion as a percentage of the total radioactive dose
Secondary	
• To evaluate the safety and tolerability of linerixibat after single IV and oral doses in healthy participants.	• Incidence and severity of adverse events. • Laboratory safety, 12-lead ECG, and vital signs parameters.
Exploratory	
• To generate samples that will be used to characterize the metabolite profile of linerixibat following a single IV microtracer dose of [^{14}C]-linerixibat concomitant with an oral dose of non-radiolabelled linerixibat (plasma, urine, feces, duodenal bile) and a single, oral dose of [^{14}C]- linerixibat (plasma, urine and feces).	• Characterization and quantification of metabolites in plasma, urine, feces, and duodenal bile (qualitative identification in bile). These analytical investigations will be conducted, and the results reported under a separate nonclinical GSK protocol.

1 For measured concentrations of linerixibat in plasma, the nomenclature **[^{14}C]-linerixibat** describes the parent linerixibat concentration derived via analysis by liquid chromatography (LC) + Accelerator Mass Spectrometry (AMS), whereas **linerixibat** describes the parent linerixibat concentration derived via liquid chromatography-tandem mass spectrometry (LC/MS/MS).

Overall Design:

The aim of the study is to assess pharmacokinetics (PK), balance/excretion, and metabolism of linerixibat using [^{14}C]-radiolabelled drug substance administered intravenously as a microtracer dose (concomitantly with an oral, non-radiolabelled dose)

and [^{14}C]-radiolabelled drug substance administered orally. Safety data will include AE reporting, 12-lead ECG, vital signs, and laboratory safety tests. Blood will be sampled extensively on the day of dosing, and daily until Day 8 for assessment of the PK of linerixibat and its metabolites. Duodenal bile will be collected non-invasively using a suitable nylon string device in Period 1 only.

Disclosure Statement: This is a single group, single center, [^{14}C]-linerixibat mass balance study with a two-period, single sequence and no masking.

Number of Participants: Six participants will be screened and enrolled to achieve at least 4 participants completing the two treatment periods.

If one or more participants prematurely discontinue the study, replacement participants will be enrolled at the discretion of the sponsor and in consultation with the investigator. Any replacement participants will be required to complete both treatment periods.

Intervention Groups and Duration:

Each participant will be involved in the study for up to 10 weeks. He will have a screening visit, two inpatient treatment periods (Treatment Periods 1 and 2), separated by about 7 days (at least 13 days between doses), and a follow-up visit 1-2 weeks after the last assessment in Treatment Period 2. During both treatment periods, participants will reside in the unit from the morning of Day -1 until all procedures are completed 168 h post-dose (on Day 8). Participants may be asked to stay for up to 1 week longer in Treatment Period 2, if required to demonstrate sufficient recovery of drug-related material. Thereafter, if deemed necessary to demonstrate sufficient recovery of radioactivity, participants may be asked to collect excreta at home.

Data Monitoring Committee: No

Treatment Period 1 (oral tablets and ^{14}C-intravenous infusion)

On Day 1 of Treatment Period 1, after an overnight fast of at least 8 h, each participant will take a single 90 mg oral dose of linerixibat; participants will continue to fast for 2 h after dosing. Immediately after the oral dose, the IV infusion of 100 µg of [^{14}C]-linerixibat (approximately 9.25 kBq; 250 nCi) will begin and continue over 3 h. Blood samples, all voided urine, and feces will be collected for 168 h after oral dosing (until Day 8), while duodenal bile will be collected as described below. Participants will be discharged on study Day 8 after completion of the 168-h sample collection and other planned assessments.

A non-invasive device to collect duodenal bile will be used only in Treatment Period 1. The collection string and small weight will be swallowed 3.5 h before the oral dose/start of IV infusion, a duration recommended to allow transit of the string to the duodenum, while participants are in a fasted state. It will be removed about 3 h after the oral dose/start of IV infusion (immediately following the end of the 3 h IV infusion), a time when the oral dose is expected to have transitioned from the stomach to the duodenum. At 2 h after the start of IV infusion (i.e., 1 h before string withdrawal), a food cue (small standard high-fat meal) will be given to stimulate gall bladder emptying.

Treatment Period 2 (oral solution)

On Day 1 of Treatment Period 2, after an overnight fast of at least 8 h, each participant will receive 90 mg [^{14}C]-linerixibat (approximately 4.96 MBq; 134.1 µCi) as an oral solution; this is considered 0 h for sample collection. Participants will continue to fast for 2 h after dosing, when the same small standard high-fat meal as in Period 1 will be given.

Blood, urine and fecal samples will be collected for a minimum of 168 h after dosing, (seven 24-h samples, which will complete on Day 8). Radioactivity quantification using liquid scintillation counting (LSC) will be performed on each 24-h urine collection and each 24-h fecal homogenate. Criteria for discharge for Period 2 are based on demonstrated cumulative recovery of radioactivity >90% or <1% in two consecutive samples (see Section 4.1.4). The inpatient stay may be extended by up to 7 days to meet the discharge criteria. In the unlikely event that excretion is still >1% in the 24-h collection period prior to discharge on Day 15, the participant will continue to collect fecal samples only, at home, at 24-h intervals. Samples will be returned to the unit every 2 to 3 days for analysis.

Total Radiation Exposure

The total amount of radiation exposure in the study is 4.97 MBq (134.3 µCi).

1.2. Schema

Figure 1 Study Intervention Schematic

Up to 30 days	*9-day inpatient stay*	*At least 13 days between doses*	*~9-day inpatient stay*		*1-2 weeks later*
Screening	**Treatment Period 1**		**Treatment Period 2**		**Follow-up**
	Single IV microtracer dose of 100 µg [^{14}C]-linerixibat concomitantly with 90 mg dose (two 45 mg tablets) of non-radiolabelled GSK-2330672		Single oral 90 mg dose of [^{14}C]-linerixibat solution *Radioactivity Recovery Checked*[a]		

[a] In Period 2, on Day 8, radioactivity recovery will be evaluated for the first six samples and sample collection for the 7th sample will complete.

- If >90% of the administered radioactivity has been recovered in samples from Day 1-6 , then the participant will be discharged on Day 8.
- If ≤90% has been recovered, then the results of the 7th sample (144-168 h sample) will be included in evaluation on Day 9.
- *On Day 9*, if >90% of radioactivity has been recovered or <1% has been excreted on two consecutive days (Day 6 and Day 7 samples), then the participant may be discharged. Otherwise, the participant will remain in the clinical unit and 24-hr samples will continue to be collected and radioactive recovery re-evaluated on a daily basis until meeting the criteria for discharge.
- All subjects will be discharged on or before Day 15. If radioactive recovery criteria have not yet been met, then subjects will continue to collect samples at home and return to the clinical unit for assay.

1.3. Schedule of Activities (SoA)

The timing and number of planned study assessments, including safety and pharmacokinetic assessments, may be altered during the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring. Participants who withdraw from the study early should be subject to those assessments that would be required at discharge in that treatment period, if participants agree.

Any changes in the timing or addition of time points for any planned study assessments must be documented and approved by the relevant study team member and then archived in the sponsor and site study files but will not constitute a protocol amendment. The Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the Informed Consent (ICF).

If assessments are scheduled for the same nominal time, the assessments should occur in the following order:

1. vital signs
2. 12-lead ECG
3. blood draws
4. other assessments

Note: The timing of the assessments should allow the blood draw to occur at the exact nominal time.

Table 1 SoA: Treatment Period 1 (oral tablets and intravenous infusion)

Visit	Screening[1]	Day -1	Pre-dose (incl. 0h)	Treatment Period 1																			
Day	-30 to -2	-1	1													2		3	4	5	6	7	8
Hrs Post-dose Procedure			-3.5	0	0.5	1.0, 1.5	2.0, 2.5	3.0, 3.5	4.0, 4.5	5.0, 5.5	6	8	10	12	24	36	48	72	96	120	144	168	
Informed consent	X																						
Adm. to unit pre-tx		X																					
Discharge from unit[2]																							X
Medical history (including drug/alcohol use)	X																						
Demography	X																						
12-lead ECG [3]	X																						X
Vital signs [4]	X	X	X						X														X
Drugs of abuse screen	X	X																					
Alcohol, cotinine, & CO breath tests	X	X																					
HIV and hepatitis B and C screen	X																						
Laboratory safety tests (incl. LFTs)	X	X																					X
Physical exam [5]	X-full	X																					X
String bile collection test initiated [6]			X																				
Oral linerixibat [7]				X																			
14C-linerixibat IV				 3h																			
Monitor local tolerability (IV)				 3h																			

SoA: Treatment Period 1 (oral tablets and intravenous infusion) (Table 1 continued)

Visit	**Screening**[1]	**Day -1**	**Pre-dose (incl. 0h)**	**Treatment Period 1**																			
Day	**-30 to -2**	**-1**	1													2		3	4	5	6	7	8
Hrs Post-dose **Procedure**			***-3.5***	***0***	***0.5***	***1.0, 1.5***	***2.0, 2.5***	***3.0, 3.5***	***4.0, 4.5***	***5.0, 5.5***	***6***	***8***	***10***	***12***	***24***	***36***	***48***	***72***	***96***	***120***	***144***	***168***	
Standard small high fat meal[8]							X-2h																
String bile collection device removed[9]								X 3h															
Blood samples for plasma radioactivity[10]	X	X		X	X	X, X	X, X	X, X	X, X	X, X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood samples for plasma drug assay[10]				X	X	X, X	X, X	X, X	X, X	X, X	X	X	X	X	X	X	X	X	X	X	X	X	
Blood samples for plasma metabolites[11]						X 1h	X 2h	X 3h			X 6h												
Genetic sample		X																					
Fecal collection[12]	Day -2 or -1				←										→								
Urine collection[12]			X		←										→								
AE/SAE/Concom-itant med. review[13]	←																						→

Abbreviations: AE: adverse event; ECG: electrocardiogram; HIV: human immunodeficiency virus; IV: intravenous; LFTs: liver function tests; PGx: pharmacogenetics; Pre-tx: pre-treatment; SAE: serious adverse event.

Notes:

[1] Screening will be within 30 days before Day 1.

[2] Participants will be discharged for a washout period prior to dosing in Treatment Period 2; there will be at least 13 days between a participant's oral doses. Participants who withdraw from the study early should be subject to those assessments that would be required at discharge in that treatment period, if participants agree.

[3] Single ECG measurements will be obtained. If any measurement is considered abnormal, then two additional ECGs will be taken. The mean of the triplicate measurements will be used. The Screening measurement will be evaluated for eligibility and used as baseline.

[4] Triplicate measurements of heart rate and systolic and diastolic blood pressure; single measurements of tympanic temperature and respiratory rate.

[5] A full physical exam should be performed at baseline, including at minimum: assessments of the eyes, skin, joints, and the cardiovascular, respiratory, gastrointestinal and neurological systems; height, and weight should be measured and recorded, and BMI calculated and recorded. Brief physical exams should be performed at later visits, including at minimum: assessments of the skin, lungs, cardiovascular system, abdomen (liver and spleen) and weight.

[6] The string bile collection device will be swallowed 3.5 h before the oral dose/start of IV infusion, while participants are in a fasted state.

[7] Participants will fast for at least 8 h before oral dosing and continue to fast for 2 h after oral dosing.

[8] Meal time is specified for Day 1 mid-day only: approximately 2h (i.e., 2h ± 15 mins) after oral dose/start of IV infusion to stimulate gall bladder emptying. On all other days, meals will be served at the unit's standard times.

[9] The string bile collection device will be removed 3 h after the oral dose/start of IV infusion (immediately after the end of the IV infusion).

[10] Background radiation will be measured in plasma samples obtained from blood collected at screening, on admission (Day –1), and pre-dose on Day1. Total radioactivity, [^{14}C]-linerixibat analysis, and linerixibat analysis will be performed for all post-dosing plasma samples. All pre-dose plasma samples should be obtained from blood taken immediately prior to the oral dose/start of IV infusion; sampling times are relative to the oral dose on Day 1, unless otherwise indicated.

[11] Plasma samples for metabolite profiling will be obtained from blood samples collected on Day 1 at 1h, 2h, 3h, and 6h post-dosing.

[12] Urine and feces will be collected at pre-dose (up to 3 h pre-dose for the urine sample; up to 24 h pre-dose for the fecal sample). Starting at the time of oral dosing, all urine and feces will be collected as seven 24-h samples throughout Period 1 (total of 168 hours post-dose) in addition to pre-dose samples.

[13] AEs will be collected from the start of study intervention until the final follow-up visit. All SAEs will be recorded from the time each participant consents to participate in the study,

Table 2 SoA: Treatment Period 2 (oral solution)

Visit	**Day -1**	**Pre-dose (incl. 0h)**	**Treatment Period 2**																		**F/up**
Day	**Day -1**	1												2		3	4	5	6	7	**8**[1]	14 – 21[2,3]
Hrs Post-dose **Procedure**		*0*	*0.5*	*1*	*2*	*3*	*4*	*5*	*6*	*8*	*10*	*12*	*24*	*36*	*48*	*72*	*96*	*120*	*144*	*168*		
Admission to unit	X																					
Discharge from unit [4]																					X [4]	
12-lead ECG [5]		X																			X	(X)
Vital signs [6]	X	X					X														X	X
Drugs of abuse screen	X																					
Alcohol, cotinine, & CO breath tests	X																					
Laboratory safety tests (including LFTs)	X																				X	(X)
[^{14}C]-linerixibat oral solution		X																				
Brief physical exam [7]	X																				X	X
Standard small high fat meal [8]					X																	
Blood samples for plasma radioactivity [9]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood samples for plasma drug assay [9]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Blood samples for plasma metabolites [10]				X	X	X			X													
Urine collection[11]		X	←																	→		
Fecal collection[11]		X	←																	→		
AE/SAE/concomitant medication review [12]	←																					→

Abbreviations: AE: adverse event; ECG: electrocardiogram; FU: follow-up; HIV: human immunodeficiency virus; LFTs: liver function tests; SAE: serious adverse event; (X): at follow-up visit, these assessments would only be performed to follow-up a result from the day of discharge.

Notes:

[1] If discharge does not occur on Day 8, the following assessments should be performed on the day of discharge instead of Day 8: vital signs, 12-lead ECG, brief physical examination.

[2] The follow-up will be between 7–14 days after the participant's last assessment in Treatment Period 2.

[3] Participants who withdraw from the study early should be subject to those assessments that would be required at discharge in that treatment period, if participants agree.

[4] Discharge from Period 2 will be contingent upon demonstration of adequate recovery of radioactivity (see Section 4.1.4).

[5] Single ECG measurements will be obtained. If any measurement is considered abnormal, then two additional ECGs will be taken, and the mean of the triplicate measurements used. The Screening measurement will be evaluated for eligibility and used as baseline.

[6] Triplicate measurements of heart rate and systolic and diastolic blood pressure; single measurements of tympanic temperature and respiratory rate.

[7] Brief physical exams should include at minimum assessments of the skin, lungs, cardiovascular system, abdomen (liver and spleen), and weight.

[8] Participants will fast for at least 8 h before oral dosing and continue to fast for 2 h after dosing. Meal time is specified for approximately 2h (i.e., 2h ± 15 mins) after the oral dose on Day 1, when the same small standard high fat meal as in Period 1 will be provided. On all other days, meals will be served at the unit's standard times.

[9] Total radioactivity, [^{14}C]-linerixibat analysis, and linerixibat analysis will include predose and all post-dosing samples: at 0.5h post-dose, then every hour through 8h, then at 10h, 12h, 24h, 36h, 48h, and then daily until discharge. All pre-dose samples should be obtained immediately prior to the oral dose; sampling times are relative to the oral dose on Day 1, unless otherwise indicated.

[10] Plasma samples for metabolite profiling will be obtained from blood samples collected on Day 1 at 1h, 2h, 3h, and 6h post-dosing.

[11] Urine and feces will be collected at pre-dose (up to 3 h pre-dose for the urine sample; up to 24 h pre-dose for the fecal sample). Starting at the time of oral dosing, all urine and feces will be collected as 24-h samples throughout Period 2 (at minimum for168 hours post-dose) in addition to pre-dose samples. If subjects are required to stay after 168 h post-dose (Day 8), collections will continue at 24-h intervals (see Section 4.1.4).

[12] AEs and SAEs will be collected until the final follow-up visit.

2. INTRODUCTION

Linerixibat is an oral small molecule inhibitor of the human ileal bile acid transporter (IBAT) that is located on the brush border of enterocytes lining the terminal ileum. Linerixibat is a highly soluble molecule, designed for gastrointestinal (GI) retention and minimal systemic exposure. It has the potential to improve symptoms of pruritus in cholestatic liver disease, such as primary biliary cholangitis (PBC).

2.1. Study Rationale

This study will assess the pharmacokinetics, balance/excretion, and metabolism of linerixibat in humans using [^{14}C]-radiolabelled drug substance administered as an intravenous (IV) infusion and orally. [^{14}C]-linerixibat administered by IV infusion will be a microtracer dose; therefore, it will be administered concomitantly with an oral non-radiolabelled dose, to ensure that the pharmacokinetics (PK) are representative of a clinically-relevant dose. Use of a string bile collection device for sampling duodenal bile after IV [^{14}C]-linerixibat infusion will enable a qualitative assessment of drug metabolites in this matrix to characterise biliary elimination pathways.

Absorption, metabolism and excretion of linerixibat have been studied in pre-clinical animal models, in vitro, and in previous clinical trials. However, no dedicated clinical studies of drug absorption, metabolism, and excretion have been conducted for linerixibat. Using radiolabelled linerixibat with Accelerator Mass Spectrometry (AMS) will enable quantitative measurement of linerixibat concentrations and comprehensive identification and quantification of drug metabolites that would not otherwise be possible.

2.2. Background

Linerixibat belongs to a class of drugs that inhibit the ileal bile acid transporter. Located in the distal ileum of the gastrointestinal tract, IBAT is responsible for the active reuptake of bile acids from the gut lumen that are then returned to the liver via the portal vein, resulting in efficient enterohepatic conservation of bile acids, and negative feedback regulation of hepatic bile acid synthesis. No other IBAT inhibitors have been investigated as treatment for pruritus associated with primary biliary cholangitis (PBC).

Because IBAT, the pharmacological target of linerixibat, is located on the brush border of enterocytes in the intestinal lumen, the investigational product was designed to have low permeability and high polar surface area, which limits absorption into the portal or systemic circulation.

A detailed description of the chemistry, pharmacology, efficacy, and safety of linerixibat is provided in the Investigator's Brochure (IB) (see GSK Document Number 2010N111289_05).

2.2.1. Pruritus associated with primary biliary cholangitis

PBC is a rare, chronic condition caused by the inflammatory destruction of the intrahepatic bile ducts giving rise to impaired bile acid flow and retention in the liver, which leads to hepatic scarring, fibrosis and ultimately cirrhosis and liver failure. Pruritus

is a major symptom associated with PBC. PBC pruritus is an intense itching that can occur anywhere on the body, often resulting in disturbed sleep and having a significant impact on a patient's quality of life. Currently there is great unmet medical need, with few therapies that provide limited relief of pruritus associated with PBC.

For patients with PBC, inhibition of IBAT by linerixibat is anticipated to increase excretion of bile acids, reduce bile acid concentrations in the liver and systemic circulation, and result in reduced pruritus and associated symptoms.

2.2.2. Linerixibat (GSK2330672)

Linerixibat was first administered to humans in June 2011. It has been evaluated in a series of nonclinical studies and clinical trials. *In vitro* and *in vivo* studies established activity of linerixibat as an inhibitor of IBAT and confirmed the molecule has minimal systemic exposure. Three Phase 1 studies in healthy participants (including one study in Japanese participants), two Phase 2 studies in Type 2 diabetic (T2D) patients, and one Phase 2 study in patients with pruritus secondary to PBC have been completed.

In these studies, linerixibat, did not result in any findings during safety monitoring that would preclude conduct of planned short-term clinical trials in patient populations with T2D or PBC. However, the high frequency of AEs of diarrhoea among T2D participants taking metformin 850 mg BID contributed to the decision to terminate development for this condition.

In a randomized, placebo-controlled, 14-day cross-over study (BAT117213), linerixibat was given as 45 mg BID on Days 1 through 3 and as 90 mg BID on Days 4 through 14. Linerixibat treatment resulted in a statistically significant decrease in pruritus severity compared to placebo, along with decreases in fatigue, sleep disturbance and overall disability compared to placebo. No serious adverse events (SAEs) were reported. Diarrhoea was the most frequent adverse event (AE) during treatment with linerixibat: seven with linerixibat vs one with placebo (Hegade, 2017).

A Phase 2 dose-ranging study in participants with pruritus secondary to PBC is currently ongoing and will inform further development in PBC pruritus (see GSK IB, Section 1.1).

Detailed information relating to non-clinical pharmacology, safety pharmacology, PK and metabolism, toxicology and other pre-clinical data can be found in the linerixibat IB.

2.3. Benefit/Risk Assessment

The potential clinically important risks of study intervention and trial procedures, and their mitigation, are given in Appendix 5 (see Section 10.5).

More detailed information about the known and expected benefits, risks, and reasonably expected AEs associated with linerixibat may be found in the IB, Section 6.

2.3.1. Risk Assessment

Potential risks of clinical significance, relevant supporting data and rationale, and mitigation strategy are presented in Appendix 5 (see Section 10.5).

Justification of the use of healthy participants below the age of 50 years is required in accordance with the guidelines of the Administration of Radioactive Substances Advisory Committee (ARSAC) and the Association of the British Pharmaceutical Industry, as well as GSK internal policies. The assessment of the radiation dose to male participants from the oral and IV administration of the study intervention is within the lower end of exposure defined by the World Health Organization's recommendation as acceptable for the general public (Public Health England, Appendix 6 [see Section 10.6]). Therefore, enrolling healthy male participants between 30 and 55 years in this study is acceptable.

2.3.2. Benefit Assessment

The healthy participants will receive no clinical benefit for participation in this study.

2.3.3. Overall Benefit: Risk Conclusion

Overall, the available data from non-clinical and clinical studies have not identified prohibitive risks associated with linerixibat at the exposures planned for this study. While there are a number of important potential risks identified for linerixibat and the study procedures, these can be addressed in this clinical trial with proper participant selection, close safety monitoring, and specific risk characterisation and mitigation (Appendix 5 [see Section 10.5]).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
• To determine parent linerixibat and total drug-related radioactivity systemic concentrations following a single IV microtracer dose of [^{14}C]-linerixibat (concomitantly with an oral dose of non-radiolabelled linerixibat) and oral dose of [^{14}C]-linerixibat[1]	• AUC(0–inf), AUC(0–t), C_{max}, t_{max} and $t_{1/2}$ of parent and total drug-related material (radioactivity) in plasma. • Volume (Vss) and clearance (CL) of parent after IV dose (Period 1 only). • Hepatic clearance (CLh) will be estimated from IV clearance (CL_{IV}) and renal clearance (CLr)
• To estimate the oral bioavailability of linerixibat	• Direct estimation of F (absolute oral bioavailability), indirect calculation of Fh (fraction of drug escaping first pass hepatic clearance), and Fa (fraction absorbed)
• To determine the rate and extent of excretion of total radioactivity in urine and feces and the total recovery of radioactivity, following IV and oral administration of [^{14}C]-linerixibat	• Urinary and fecal cumulative excretion as a percentage of the total radioactive dose
Secondary	
• To describe the safety and tolerability of linerixibat after single IV and oral doses in healthy participants.	• Characterize observed adverse events, and abnormal laboratory, 12-lead ECG, and vital signs assessments observed.
Exploratory	
• To generate samples that will be used to characterize the metabolite profile of linerixibat following a single IV microtracer dose of [^{14}C]-linerixibat concomitantly with an oral dose of non-radiolabelled linerixibat (plasma, urine, feces, duodenal bile) and a single, oral dose of [^{14}C]-linerixibat (plasma, urine and feces).	• Characterization and quantification of metabolites in plasma, urine, feces, and duodenal bile (qualitative identification in bile). These analytical investigations will be conducted, and the results reported under a separate GSK nonclinical protocol.

AUC(0–inf): Area under the plasma concentration-time curve from time zero (pre-dose) extrapolated to infinite time, AUC(0–t): Area under the plasma concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a participant across all treatments, Cmax: Maximum observed plasma concentration, tmax: time of occurrence of Cmax, t1/2: terminal phase half-life. ECG: Electrocardiogram.

[1] For measured concentrations of linerixibat in plasma, the nomenclature **[^{14}C]-linerixibat** describes the parent linerixibat concentration derived via analysis by liquid chromatography (LC) + Accelerator Mass Spectrometry (AMS), whereas **linerixibat** describes the parent linerixibat concentration derived via liquid chromatography-tandem mass spectrometry (LC/MS/MS).

4. STUDY DESIGN

4.1. Overall Design

This is a single group, single center, [^{14}C]-linerixibat mass balance study with a two-period, single sequence, and no masking.

This open-label, single-center, non-randomised, 2-period, single-sequence, mass balance study will enroll a cohort of 6 healthy male participants. The aim of the study is to assess the pharmacokinetics, balance/excretion, and metabolism of linerixibat using [^{14}C]-radiolabelled drug substance administered as an IV infusion of a microtracer dose (concomitant with an oral, non-radiolabelled dose) and orally.

Four to six healthy participants are deemed sufficient. To minimise the number of exposed to radiation, participants who discontinue early will not be replaced unless the total number of participants who complete dosing and all critical assessments drops below 4.

Each participant will be involved in the study for up to 10 weeks. He will have a screening visit, two treatment periods (Treatment Periods 1 and 2), separated by about 7 days (at least 13 days between oral doses), and a follow-up visit 1-2 weeks after the last assessment in Treatment Period 2. During both treatment periods, participants will reside in the unit from the morning of Day -1 until all procedures are completed at 168 h post-dose (on Day 8). Participants may be asked to stay for up to 1 week longer in Treatment Period 2, if excretion of drug-related material takes longer than anticipated (see Figure 1).

Safety data will include AE reporting, 12-lead ECG, vital signs, and laboratory safety tests. Blood will be sampled extensively on the day of dosing and daily until Day 8, for assessing the PK of linerixibat and metabolites.

4.1.1. Screening Period

Participants must be screened within 30 days before the first dose of linerixibat and must meet all eligibility criteria.

The following demographic parameters will be captured: year of birth, sex, race and ethnicity.

Medical/medication/family history will be assessed as related to the inclusion/exclusion criteria listed in Section 5.

Baseline is defined as the value for clinical laboratory, ECG, VS, and physical exam results that is collected closest to but before the first dose on Day 1 in Period 1.

4.1.2. Treatment Period 1 (oral tablets and intravenous infusion)

On Day 1 of Treatment Period 1, after an overnight fast of at least 8 h, each participant will have a blood sample collected for time 0h, immediately followed by the single 90 mg oral dose of linerixibat and the IV infusion of 100 µg of [^{14}C]-linerixibat (approximately 9.25 kBq; 250 nCi). Participants will continue to fast for 2 h after dosing, and the IV

infusion will continue over 3 h. All voided urine and feces will be collected in seven 24-h samples for a total of 168 h after oral dosing (until the morning of Day 8). The last blood samples, vital signs, ECG, and brief physical examination of the treatment period will be obtained on Day 8. Duodenal bile will be collected on Day 1, as described below.

A non-invasive device to collect duodenal bile will be used only in Treatment Period 1. The bile collection string and a small weight will be swallowed 3.5 h before the oral dose/start of IV infusion, a duration recommended to allow transit of the string to the duodenum, while participants are in a fasted state. It will be removed about 3 h after the oral dose/start of IV infusion (immediately following the end of the 3 h IV infusion), a time when the oral dose is expected to have transitioned from the stomach to the duodenum. At 2 h after the start of IV infusion (i.e., 1 h before string withdrawal), a food cue (small standard high-fat meal) will be given to stimulate gall bladder emptying.

Participants will be discharged on study Day 8 after completion of the 168-h sample collection and completion of other scheduled assessments.

4.1.3. Treatment Period 2 (oral solution)

On Day 1 of Treatment Period 2, after an overnight fast of at least 8 h, each participant will have a blood sample collected for time 0h, immediately followed by the 90 mg [^{14}C]-linerixibat (approximately 4.96 MBq; 134.1 µCi) dose as an oral solution. Participants will continue to fast for 2 h after dosing, when the same small standard high-fat meal as in Period 1 will be provided. Urine and fecal samples will be collected for a minimum of 168 h after dosing (Day 7 sample completing on Day 8). Radioactivity quantification using LSC will be performed on each of the 24-h urine collections and 24-h fecal homogenates.

4.1.4. Demonstration of radioactivity recovery before discharge (Period 2)

LSC radioquantification results from samples collected for Days 1 through 6 (through 144 h post-dose) will be reviewed on Day 8. The GSK In vitro / In vivo Translation (IV/IVT) Study Monitor should be consulted to agree with release of a participant. This process is outlined in detail in the Study Reference Manual (SRM).

- If >90% of the administered radioactivity has been recovered from the Day 1-6 samples, then the participant may be discharged on Day 8 after all scheduled assessments have been completed, including complete collection of Day 7 urine and feces samples (24 h collection period ending on Day 8).

- If ≤90% has been recovered, then the radioquantification results will be re-evaluated on Day 9 with Day 7 results included.

On Day 9, if >90% has been recovered or <1% of the dose has been excreted on both Day 6 (120-144 h) and Day 7 (144-168 h), then the participant may be discharged on Day 9.

If the participant is not eligible for discharge on Day 9, or if the results are inconclusive, the participant will remain at the unit. Urine and fecal collections will continue at 24-h

intervals for up to 7 additional days (until the morning of Day 15). Once <1% of the dose is recovered in 2 consecutive 24-h periods where samples are provided (and the samples were of a sufficient size to make this assessment), or once >90% of the radioactivity has been recovered, the participant will be discharged.

All remaining participants will be discharged from the unit no later than Day 15. In the unlikely event that excretion is still ≥1% in the 24-h collection period prior to discharge on Day 15, the participant will continue to collect fecal samples only, at home, over 24-h intervals. Samples will be returned to the unit every 2 to 3 days for analysis.

4.1.5. Follow-up

Follow-up procedures will be done 7-14 days after the participant's last assessment in Treatment Period 2. The follow-up period may be extended if:

(i) radioactivity excretion is still higher than 1%;

(ii) a participant has an unresolved AE at the follow-up visit, which, in the opinion of the investigator, merits further follow-up; or

(iii) new information becomes available that supports an extended follow-up period.

The investigator and GSK IV/IVT Monitor and/or GSK Medical Monitor as appropriate will agree on the nature of the extended follow-up. For example, participants may have a telephone follow-up at which they are asked about AEs, or participants may be asked to attend extra outpatient visits for additional monitoring of plasma radioactivity levels and/or for extra safety tests. The extra safety tests might include tests that are not described in this protocol. The investigator reserves the right, during or after the study, to repeat safety tests or to do any extra safety tests that are in the best interest of the participants. Those extra tests may or may not be described in this protocol.

4.2. Scientific Rationale for Study Design

In the first treatment period of this study, an IV microtracer dose of [^{14}C]-linerixibat will be infused over 3 h, beginning concurrently with an oral dose of non-radiolabelled linerixibat. The non-radiolabelled 90 mg dose of linerixibat is to ensure that the PK of the microdose represents a therapeutically relevant total body exposure to the drug. In the second treatment period, a 90 mg oral solution of [^{14}C]-linerixibat will be used for comparison.

Biliary elimination pathways will be characterised after the IV dose by inclusion of duodenal bile collection in Period 1. Complexities in human fecal sample analysis such as extraction, stability in the gastrointestinal tract, and endogenous contamination are minimised through assessment of the metabolite profile in duodenal bile after IV drug administration.

4.3. Justification for Dose

In Study BAT117213 (GSK Document Number 2015N268599_01), PBC participants received linerixibat at a dose of 45 mg twice daily increased to 90 mg twice daily after 3 days and continued for 14 days in total. Based on responses observed in these PBC participants and in relevant biomarkers (total serum bile acids and 7-α-hydroxy-4-cholesten-3-one [C4]) measured in Type II diabetic (T2DM) participants, the currently expected therapeutic dose is 90 mg twice daily. Further details of the findings in these studies are provided in the IB.

This 90 mg dose has adequate nonclinical toxicology cover for local gastrointestinal tract exposure (based on administered oral dose, i.e., 1.8 mg/kg/day assuming 50 kg body weight): 556-fold No Observed Adverse Effect Level (NOAEL) in the rat and 278-fold NOAEL in the dog for local gastrointestinal tract exposure (1000 mg/kg/day and 500 mg/kg/day dose, respectively). Further information on nonclinical toxicology studies can be found in the IB.

The 100 µg microtracer dose administered as a 3-h IV infusion is projected (based on allometry) to achieve a linerixibat Cmax <0.5 ng/mL, which is >13-fold lower than the highest observed human Cmax following a 180 mg dose (Study 205808). This dose can be categorised as a microdose and as such required safety cover is detailed in ICH M3 (R2) (International Conference on Harmonisation, 2009).

Preclinical toxicology species provide safety cover for systemic exposure to linerixibat. In vitro studies using hepatocytes from nonclinical species and humans show that there is generally no qualitative difference in metabolites formed. Preliminary non-radiolabelled studies detected no linerixibat metabolites in human plasma and urine following 90 mg BID dosing for two weeks (see IB, Section 1.1).

4.3.1. Linerixibat Oral Dose (Periods 1 and 2)

The oral linerixibat dose is within the therapeutic range for the treatment of PBC pruritus. The 90 mg dose is half the highest once-daily dose currently under investigation in the ongoing Phase 2 study (180 mg), which is also the highest dose in clinical experience (see).

4.3.2. Linerixibat Intravenous Dose (Period 1)

The dose of [^{14}C]-linerixibat to be administered intravenously is a microdose of 100 µg, which will be infused over 3 h. A microdose was selected because linerixibat has not previously been administered by IV infusion to humans. That dose level meets the criterion for a microdose, for the following reasons:

- It is ≤ 100 µg.
- It is ≤ 1/100th of the lowest pharmacologically-active oral dose in healthy participants with normal renal function (i.e., 10 mg single, oral dose) based on an increase in serum C4 (see GlaxoSmithKline Document Number 2016N306458_00 Study ID 205808).

- It is ≤ 1/100,000th the no observed adverse effect level (NOAEL) of 500 mg/kg/day, the highest dose given in the 13-week dog study, where no adverse effects were observed.

Systemic exposure (based on Cmax) at this NOAEL dose was >13-fold the maximum observed concentration of 6.64 ng/mL at a clinical dose of 180 mg/day in Study 205808.

To ensure clinically-relevant systemic exposure during the microdose, the IV infusion of [^{14}C]-linerixibat will be administered concomitantly with an oral non-radiolabelled dose of 90 mg linerixibat.

The 100 µg microtracer dose administered as a 3-h IV infusion is projected (based on allometry) to achieve a linerixibat Cmax <0.5 ng/mL, which is >13-fold lower than the highest observed human Cmax following a 180 mg dose (Study 205808). This dose can be categorised as a microdose and as such required safety cover is detailed in ICH M3 (R2).

4.3.3. Radiolabel Dose

The effective dose of radiolabelled drug administered in human mass balance studies is calculated from data on the distribution and elimination of the radioactive drug from laboratory animals, considering the nature of the isotope, the concentration of radioactivity in individual tissues/organs and the residence or elimination half-life of the radioactivity from those tissues/organs.

4.3.4. Total Radiation Exposure

In this study, each participant will receive the following doses of radioactivity:

- approximately 9.25 kBq (250 nCi) in Treatment Period 1.
- approximately 4.96 MBq (134.1 µCi) in Treatment Period 2.

The total amount of radiation exposure in the study is 4.97 MBq (134.3 µCi).

It is estimated that the combined total effective dose for the two treatment periods will be <1mSv. On this basis, the maximum administered activity would comply with the ICRP, 1992 recommendation of a 1 mSv maximum for Category IIa projects (further details are in Appendix 6 [see Section 10.6]).

4.4. End of Study Definition

A participant is considered to have completed the study if he has completed all phases of the study including the follow-up visit (end of study visit).

The end of the study is defined as the date of the last visit of the last participant in the study.

5. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

Age

1. Aged 30 to 55 years, inclusive, at the time of signing the informed consent.

Type of Participant and Disease Characteristics

2. Healthy, as determined by the investigator or medically qualified designee, based on a medical evaluation including medical history, physical examination, vital signs, laboratory tests, and ECG. A participant with a clinical abnormality or laboratory parameter (i.e., outside the reference range for the population being studied), which is not specifically listed in the eligibility criteria, may be included only if the investigator agrees and documents that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures.
3. History of regular bowel movements (averaging one or more bowel movements per day).
4. Non-smoker, or ex-smoker who hasn't regularly smoked for the 6 months before screening.

Weight

5. Body weight of 50 kg and above, and body mass index (BMI) within the range 19.0 to 31.0 kg/m2 (inclusive).

Sex

6. Male only.

   Participants must agree to use contraception as follows: participants with female partners of childbearing potential must agree to use a condom from the time of first dose of study intervention until 1 month after their last dose. Further details are given in Appendix 4 (see Section 10.4.1).

Informed Consent

7. Capable of giving signed informed consent as described in Appendix 5 (Section 10.1.3), which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

5.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones). Participants with a history of cholecystectomy must be excluded.
2. Significant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; constituting a risk when taking the study intervention ; or interfering with the interpretation of data.
3. Any clinically relevant abnormality identified at the screening medical assessment (physical examination/medical history) clinical laboratory tests, or 12-lead ECG.
4. Current episode, recent history, or chronic history of diarrhoea.
5. Lymphoma, leukemia, or any malignancy within the past 5 years except for basal cell or squamous epithelial carcinomas of the skin that have been resected with no evidence of metastatic disease for 3 years.
6. Any current medical condition (e.g. psychiatric disorder, senility, dementia, or other condition), clinical or laboratory abnormality, or examination finding that the investigator considers would put the participant at unacceptable risk, which may affect study compliance or prevent understanding of the aims or investigational procedures or possible consequences of the study.
7. Regular use of known drugs of abuse or history of drug abuse or dependence within 6 months of the study.
8. Regular alcohol consumption within 6 months prior to the study defined as an average weekly intake of >21 units. One unit is equivalent to 8 g of alcohol: a glass (~240 mL) of beer, 1 small glass (~100 mL) of wine or 1 (~25 mL) measure of spirits.
9. History of or regular use of tobacco- or nicotine-containing products in the 6 months prior to screening.

Prior/Concomitant Therapy

10. Past or intended use of over-the-counter or prescription medication, including analgesics (eg, paracetamol), herbal medications, or grapefruit and Seville orange juices within 14 days prior to the first dose of study intervention until completion of the follow-up visit.
11. Administration of any other IBAT inhibitor in the 3 months prior to screening.

Prior/Concurrent Clinical Study Experience

12. Current enrolment in a clinical trial; recent participation in a clinical trial and has received an investigational product within 3 months before the first dose in the current study.
13. Exposure to more than 4 new chemical entities within 12 months before the first dose in the current study.
14. Participation in a clinical trial involving administration of ^{14}C-labelled compound(s) within the last 12 months. A participant's previous effective dose will be reviewed by the medical investigator to ensure there is no risk of contamination/carryover into the current study.
15. Received a total body radiation dose of greater than 10.0 mSv (upper limit of IRCP category II) or exposure to significant radiation (e.g., serial x-ray or computed tomography [CT] scans, barium meal, etc.) in the 3 years before this study.

Diagnostic assessments including clinical laboratory tests, or 12-lead ECG.

16. Alanine transaminase (ALT) >1.5x upper limit of normal (ULN).
17. Bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
18. Presence of Hepatitis B surface antigen (HBsAg) at screening or positive Hepatitis C antibody test result at screening or within 3 months before the first dose of study intervention.

    Note: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA test is obtained.
19. Screening estimated glomerular filtration rate (eGFR) <45 mL/min/1.73m² based on the Modification of Diet in Renal Disease (MDRD) Study equation (Levey, 2006).
20. Positive pre-study drug/alcohol screen.
21. Urinary cotinine levels indicative of smoking.
22. Positive human immunodeficiency virus (HIV) antibody test.
23. QTcF >450 msec on ECG performed at Screening

    NOTES:

    The QTc must be the QT interval corrected for heart rate according to Fridericia's formula (QTcF).

    For purposes of data analysis, QTcF will be used as specified in the Reporting and Analysis Plan (RAP).
24. At screening or prior to the first dose, a supine blood pressure (BP) that is persistently higher than 140/90 millimeters of mercury (mmHg) taken in triplicate, unless deemed not clinically significant by the investigator.

25. At screening or prior to the first dose, a supine mean heart rate (HR) outside the range of 40–100 beats per minute, unless deemed not clinically significant by the investigator.

Other Exclusions

26. Has had an occupation which requires monitoring for radiation exposure, nuclear medicine procedures, or excessive x-rays within the past 12 months.

27. Unable to refrain from consumption of prohibited food and drinks (see Section 5.3) from 7 days before the first dose of study medication until the follow up visit.

28. Loss of more than 400 mL blood during the 3 months before screening, eg as a blood donor, or plan to donate blood or blood products in the 3 months after the end of the trial.

29. Unwillingness or inability to follow the procedures outlines in the protocol, including the use of the string bile collection device.

30. History of sensitivity to linerixibat, or their components thereof, or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation.

5.3. Lifestyle Considerations

5.3.1. Meals and Dietary Restrictions

Participants will be required to fast for at least 5 h before laboratory safety tests, and for at least 8 h before dosing in both treatment periods. In treatment period 1, participants will also be required to fast for 5.5 h after instillation of the string bile collection device (for 2 h after oral dose/start of infusion). In treatment period 2, participants will also be required to fast for 2 h after the oral dose. A standard, small high-fat meal will be provided at 2 h after the oral dose in each period. Meal times are specified in both treatment periods for Day 1, please refer to the SoA (see Section 1.3); on all other days, meals will be served at the standard times of the clinical site.

Participants will refrain from consumption of Seville oranges, grapefruit or grapefruit juice, pomelos, exotic citrus fruits, grapefruit hybrids, or their fruit juices from 7 days before the start of study intervention until the follow-up visit.

Participants will refrain from consumption of nuts, seeds, tomato skins, or other foods that may cause issues with homogenization of feces.

No water is allowed until 2 h after oral dosing (apart from rinsing the oral solution dose of [^{14}C]-linerixibat); water is allowed *ad libitum* at all other times.

Adequate hydration should be encouraged to help facilitate stool sample production. If needed, participants may consume prunes or prune juice to facilitate stool samples.

5.3.2. Caffeine, Alcohol, and Tobacco

During each treatment period, participants will abstain from ingesting caffeine- or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) from 24 h before admission until discharge from the unit.

Participants will abstain from alcohol for 24 h before the screening visit until the follow-up visit.

Participants must not be current smokers (no smoking for at least 6 months before the screening visit). Use of tobacco- or nicotine-containing products (including nicotine patches and other delivery devices such as vaporizers) will not be allowed from screening until after the final follow-up visit.

5.3.3. Activity

Participants will abstain from strenuous exercise from 3 days before screening and until their final follow-up visit. Participants may participate in light recreational activities during studies (e.g., watching television, reading).

5.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently enrolled in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may not be rescreened.

6. STUDY INTERVENTION

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study participant according to the study protocol (see Table 3).

6.1. Study Intervention(s) Administered

Table 3 Treatments administered during the study

Study Intervention Name:	**[^{14}C]-linerixibat solution for IV infusion**	**[^{14}C]-linerixibat oral solution**	**Linerixibat**
Dosage formulation:	IV solution	Oral solution	Oral tablets
Treatment Period	1	2	1
Unit dose strengths:	Strength: 4 µg/mL Dosage Level: 100 µg	Strength: 1.5 mg/mL Dosage Level: 90 mg	Strength: 45 mg tablet Dosage Level: 90 mg
Route of Administration:	IV infusion	Oral	Oral
Dosing instructions:	Administer 25 mL intravenously over 3 h immediately after the oral dose	Administer 60 mL in the fasted state in the morning.	Two tablets taken in the fasted state in the morning with 240 mL of room temperature water
Manufacturer:	Drug product: HMR	Drug product: HMR	Wuxi Apptec Co., Ltd.
Physical Description:	A clear, colourless solution free from visible particulates	A clear, colourless solution free from visible particulates	White to slightly coloured film-coated round tablet

6.2. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study intervention received and any discrepancies are reported and resolved before use of the study intervention.

Only participants enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study interventions must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

A description of the methods and materials required for preparation of [^{14}C]-linerixibat solutions for IV infusion and oral administration is provided in the SRM and/or Technical Assessment (TA). A Quality Agreement will be in place with the CRO performing the manufacturing operations.

Further guidance and information for the final disposition of unused study intervention are provided in the SRM.

Under normal conditions of handling and administration, study intervention is not expected to pose significant safety risks to site staff. Take adequate precautions to avoid direct eye or skin contact and the generation of aerosols or mists. In the case of

unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.

A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

Dose administrators must follow site-specific procedures for handling radiolabelled linerixibat.

6.3. Measures to Minimize Bias: Randomization and Blinding

This is an open-label study with no randomization and no blinding of participants, site personnel, or sponsor personnel.

Eligibility will be established during the Screening Period. A cohort of six participants will be admitted to the clinical research unit on the same day and will complete Period 1 and Period 2 on the same schedule with the same study interventions. This approach minimizes potential bias associated with temporal effects.

6.4. Study Intervention Compliance

Individual doses will be prepared for each participant from a bulk supply and will be confirmed by a second member of the study site staff.

All participants will receive their doses directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and reported in the CRF. The dose of study intervention and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study intervention. For oral doses, study site personnel will examine each participant's mouth to ensure that the study intervention was ingested. For IV doses, administration will be documented in the source documents and reported in the CRF.

6.5. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment must be discontinued by 15 days before the start of the study intervention. These must be recorded along with:

- reason for use
- dates of administration including start and end dates
- dosage information including dose and frequency.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Participants must abstain from taking prescription and non-prescription drugs (including vitamins and dietary and herbal supplements) within 14 days before the start of study intervention until completion of the follow-up visit.

Special warnings and precautions for use of linerixibat are given in the IB, Section 6.

6.6. Dose Modification

No dose modification is allowed.

6.7. Intervention after the End of the Study

Participants will not receive any additional study intervention from GSK after completion of the study because only healthy participants are eligible for study participation.

7. DISCONTINUATION OF STUDY INTERVENTION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

A participant may withdraw from the study at any time at his own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance or administrative reasons. Permanent discontinuation from study intervention is required for specific reasons (Section 7.1).

If study intervention is permanently discontinued, the participant will remain in the study for confirmation of excretion of radioactivity (see Section 4.1.4). Participants who are withdrawn from the study early should be subject to those assessments that would be required at discharge in that treatment period, if participants agree (see Section 1.3).

7.1. Discontinuation of Study Intervention

A participant must permanently discontinue study intervention for the pre-specified reasons below.

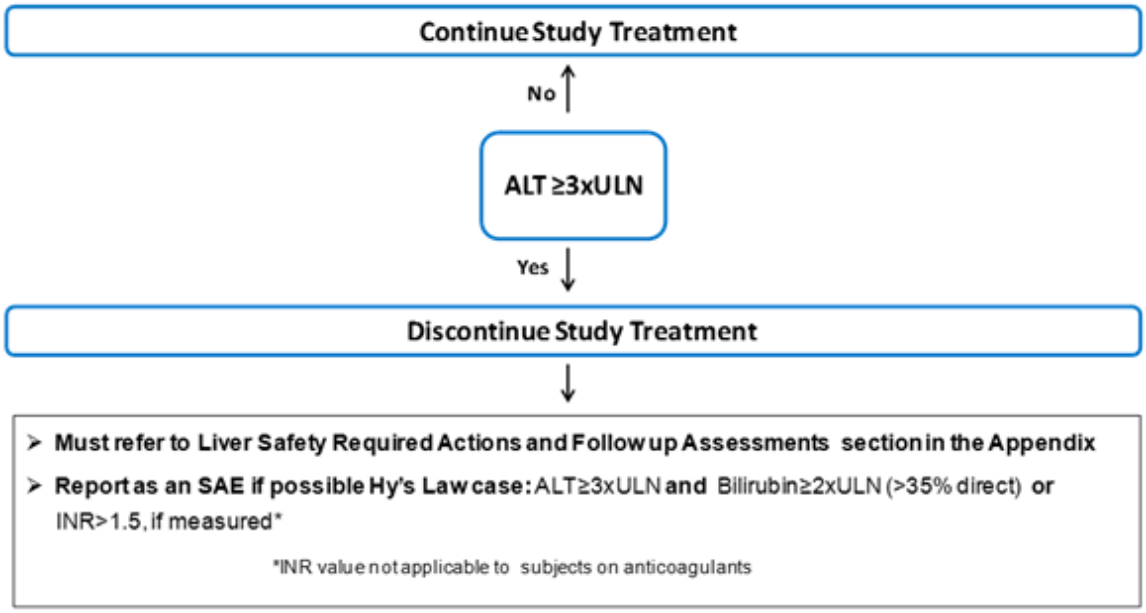
- Severe pain, redness, bleeding or swelling at the infusion site.
- Liver chemistry abnormalities exceeding the threshold criteria (see Section 7.1.1).
- QTc result meeting the stopping criteria (see Section 7.1.2)
- SAE considered related to study intervention

7.1.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology.

Study intervention will be discontinued for a participant if liver chemistry stopping criteria are met:

Figure 2 Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow up Assessments Section can be found in Appendix 8 (see Section 10.8).

7.1.1.1. Study Intervention Restart or Rechallenge after liver stopping criteria met

Study intervention restart or rechallenge after liver chemistry stopping criteria are met by any participant in this study is not allowed.

7.1.2. QTc Stopping Criteria

The QT interval will be corrected using Fridericia's formula and averaged from three ECGs obtained over a brief period to determine whether the participant should be discontinued from the study.

If an automated reading is not available, the ECG should be manually over-read by the investigator or adequately trained physician.

A participant who meets one or both bulleted criteria based on the average of triplicate ECG readings will be withdrawn from study intervention.

- QTcF >500 msec,
- Change from baseline: QTcF >60 msec

7.2. Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance or administrative reasons. Participants who withdraw from the study early should be subject to those assessments that would be required at discharge in that treatment period, if participants agree.

If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a subject withdraws from the study, he may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

The investigator will record in the source documents the results of follow-up examination of withdrawn participants, if the participant gives their consent.

7.3. Lost to Follow Up

A participant will be considered lost to follow-up if he repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he will be considered to have withdrawn from the study.

8. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA (see Section 1.3).
- Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the GSK Medical Monitor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA (see Section 1.3), is essential and required for study conduct.

- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.
- Procedures conducted as part of the participant's routine clinical management and obtained before signing the ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA (see Section 1.3).
- The timing and number of planned study assessments, including safety, and pharmacokinetic assessments may be altered during the study based on newly available data (e.g., to obtain data closer to the time of peak plasma concentrations) to ensure appropriate monitoring.
- The change in timing or addition of time points for any planned study assessments must be documented in a Note to File which is approved by the relevant GSK study team member and then archived in the study sponsor and site study files, but this will not constitute a protocol amendment.
- The IRB/IEC will be informed of any safety issues that require alteration of the safety monitoring scheme or amendment of the ICF.
- The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 600 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.
- Discontinuation of the study is handled as part of Appendix 1.

8.1. Efficacy Assessments

Efficacy of linerixibat as a treatment for PBC pruritus or any other indication is not assessed in this study.

8.2. Safety Assessments

Safety assessments include AEs, SAEs, ECGs, vital signs, and clinical laboratory tests. Specific liver test results or QTc results that require discontinuation of study intervention are presented in Section 7.1.1 and Section 7.1.2, respectively.

8.2.1. Physical Examinations

- A full physical examination will include, at a minimum, assessments of the eyes, skin, joints, and the cardiovascular, respiratory, gastrointestinal and neurological systems. Height and weight will also be measured and recorded, and BMI calculated and recorded.

- A brief physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen). Weight will also be measured and recorded.

- Investigators should pay special attention to clinical signs related to previous serious illnesses.

8.2.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes rest and will include single measurements of tympanic temperature and respiratory rate and triplicate measurements of systolic and diastolic blood pressure and heart rate.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- For time points when blood pressure and heart rate are collected in triplicate, there should be about a 2-minute interval between readings.
- Baseline will be defined as the mean of the 3 pre-dose measurements taken on Day 1 in Treatment Period 1.

8.2.3. Electrocardiograms

- ECG measurements will be obtained as outlined in the SoA (see Section 1.3). Full 12 lead ECGs will be recorded with the participant in a supine position. Heart rate, PR interval, QRS duration, and QT (uncorrected) interval will be measured. QTcF will be calculated (machine read or manually).
- At each time point at which ECGs are required, single ECG measurements are appropriate; however, two additional ECGs are required if the initial ECG measurement indicates prolonged QTc (i.e., QTcF $\geq$ 500 msec) using the automated or manually calculated QTcF value. The average QTcF value of all three ECGs will be used to determine eligibility.

8.2.4. Clinical Safety Laboratory Assessments

- Refer to Appendix 4 (see Section 10.4) for the list of clinical laboratory tests to be performed and to the SoA (see Section 1.3) for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically-significantly abnormal during participation in the study or within 14 days after the last dose of study intervention should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the investigator or GSK Medical Monitor.
- If such values do not return to normal/baseline within a period judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.

- All protocol-required laboratory assessments, as defined in Appendix 2 (see Section 10.2), must be conducted in accordance with the laboratory manual and the SoA (see Section 1.3).

8.3. Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Appendix 3 (see Section 10.3).

The investigator and any qualified designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or the study, or that caused the participant to discontinue the study (see Section 7).

8.3.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the signing of the informed consent form start of study intervention until the follow-up visit at the time points specified in the SoA (see Section 1.3).
- All AEs will be collected from start of study intervention until the follow-up visit at the time points specified in the SoA (see Section 1.3).
- Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF), not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 h, as indicated in Appendix 3(see Section 10.3). The investigator will submit any updated SAE data to the sponsor within 24 h of it being available.
- Investigators are not obligated to actively seek AEs or SAEs after the conclusion of study participation. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

8.3.2. Method of Detecting AEs and SAEs

- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Appendix 3 (see Section 10.3).
- Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

8.3.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until the event is

resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Appendix 3 (see Section 10.3).

8.3.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of the study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and investigators.
- Investigator safety reports must be prepared for Suspected Unexpected Serious Adverse Reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate according to local requirements.

8.3.5. Pregnancy

- Details of all pregnancies in female partners of male participants will be collected after the start of study intervention and until the participant's final visit.
- If a pregnancy is reported, the investigator should inform GSK within 24 h of learning of the pregnancy and should follow the procedures outlined in Appendix 4 (see Section 10.4.2).
- Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.4. Treatment of Overdose

For this study, any dose of linerixibat greater than 180 mg within a 24-hour period will be considered an overdose.

GSK does not recommend specific treatment for an overdose as there is no specific antidote for linerixibat. In the event of a suspected overdose, it is recommended that the appropriate supportive clinical care should be instituted, as dictated by the participant's clinical status.

In the event of an overdose, the investigator should:

1. Contact the GSK Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities as agreed with the Medical Monitor on a case-by-case basis.

3. Obtain a plasma sample for PK analysis if requested by the Medical Monitor (determined on a case-by-case basis).

4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or discontinuation will be made by the investigator in consultation with the GSK Medical Monitor based on the clinical evaluation of the participant.

8.5. Pharmacokinetics

Pharmacokinetics will be assessed in plasma, urine, and faeces. Samples collected as part of this study for metabolite profiling will be analysed under a separate nonclinical protocol. The results of those analyses will be reported separately.

8.5.1. Plasma Sample Collection

Blood samples will be collected for measurement of each of the following as specified in the SoA (Section 1.3): plasma total radioactivity, plasma [^{14}C]-linerixibat, plasma concentrations of linerixibat, and plasma metabolite profiling.

Details of PK blood sample collection (including the volumes to be collected), processing, storage and shipping procedures are provided in the SRM.

For samples used to evaluate the PK of linerixibat (and [^{14}C]-linerixibat), each plasma sample will be divided into 2 aliquots (1 each for PK and a back-up). Samples collected for analyses of linerixibat plasma concentration may also be used to evaluate safety or efficacy aspects related to concerns that may arise during or after the study.

Samples will be stored for no longer than 15 years after the end of the trial.

8.5.2. Urine Sample Collection

Urine samples will be collected over the time periods specified in the SoA (see Section 1.3). Urine samples will be used to determine total radioactivity excreted in urine and for subsequent metabolite profiling (to be conducted in a separate study). All participants will be asked to void their bladders before study intervention administration. A blank urine sample will be collected pre-dose (up to 3 h before oral dosing) in Treatment Periods 1 and 2. Details of urine sample collection, processing, storage and shipping procedures are provided in the SRM.

8.5.3. Fecal Sample Collection

Fecal samples will be collected over the time periods specified in the SoA (see Section 1.3). Fecal samples will be used to determine total radioactivity excreted in feces and for subsequent metabolite profiling (to be conducted in a separate study). A fecal sample will be collected from each participant before dosing in Treatment Periods 1 and 2 (the

pre-dose sample can be collected up to 48 h before oral dosing). Details of fecal sample collection, processing, storage and shipping procedures are provided in the SRM.

8.5.4. Bile Sample Collection

Bile samples will be collected via a non-invasive string device in Treatment Period 1 only, as specified in the SoA (see Section 1.3). The samples obtained will be used to investigate potential biliary metabolites (in a separate GSK study).

The string bile collection device comprises a gelatine capsule which contains 90 cm or 140 cm of nylon string attached to a 1 g steel weight. One end of the string is attached to the outside of the mouth before swallowing the capsule, so that it can still be retrieved. The gelatine capsule dissolves in the stomach whilst the string and weight continue to the duodenum via peristalsis.

A food cue will be used to stimulate gall bladder emptying at 2 h after the start of IV infusion, and the string will be withdrawn 1h later (IV infusion will be given over 3h). On withdrawal of the string through the mouth the steel weight separates from the string at the pyloric sphincter and is excreted in the feces. Once the string has been removed from the participant it will be frozen and shipped for metabolite profiling (to be conducted in a separate study). Full details of the bile sample collection, processing, storage and shipping procedures are provided in the SRM.

8.5.5. Sample Analysis

Total radioactivity measurements in urine samples and fecal homogenates will be determined by LSC and/or by AMS. Total radioactivity measurements from plasma derived from blood will be analysed, as appropriate, by AMS in Treatment Period 1 and by LSC and/or by AMS, for Treatment Period 2, as detailed in the SRM.

[^{14}C]-linerixibat and linerixibat plasma concentrations will be analysed, as appropriate, as detailed in the SRM.

Aliquots of plasma, urine, fecal homogenates and duodenal bile will be provided for metabolite analysis. Metabolite analysis will be performed and reported separately, under a separate nonclinical GSK study.

Analysis of all samples (plasma, urine, feces, and duodenal bile) will be performed under the control of Bioanalysis, Immunogenicity and Biomarkers (BIB) and Mechanistic Safety and Disposition (MSD), GlaxoSmithKline, the details of which will be included in the SRM. Raw data will be archived at the bioanalytical site (detailed in the SRM).

8.6. Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

8.7. Genetics

A 6 mL blood sample for DNA isolation will be collected, prior to the first dose, from participants who have consented to participate in the genetics analysis component of the study. Participation is optional. Participants who do not wish to participate in the genetic research may still participate in the study.

See Appendix 7 (Section 10.7) for Information regarding genetic research]. Details on processes for collection and shipment and destruction of these samples can be found in the SRM.

8.8. Biomarkers

Biomarkers are not evaluated in this study.

8.9. Medical Resource Utilization and Health Economics

Medical Resource Utilization and Health Economics parameters are not evaluated in this study.

9. STATISTICAL CONSIDERATIONS

Final analyses will be performed after the completion of the study and final dataset authorization.

9.1. Statistical Hypotheses

This study is not designed for statistical testing and therefore has no formal statistical hypothesis. The primary objectives and associated endpoints are presented in Section 1.1 and Section 3.

9.2. Sample Size Determination

No formal sample size calculation has been performed for this study. The primary objective of the study is to gain a better understanding of the compound's pharmacokinetic, excretory, and metabolic profile and 4 to 6 participants are deemed sufficient for this purpose. Six participants will be enrolled into the study. To minimise the number of participants exposed to radiation, those participants that discontinue early will not be replaced unless the total number of participants who complete both Periods 1 and 2 drops below 4.

9.3. Populations for Analyses

For purposes of analysis, the following populations are defined:

Screened Population	All participants who sign the ICF. This will be the population for reporting screened population data.

All Participants Population	All participants who take at least 1 dose of study intervention. Participants will be analysed according to the treatment they received. This will be the population for reporting safety and study population data.
Pharmacokinetic Population	All participants whom sufficient data are available to calculate the derived pharmacokinetic parameters on an as-treated basis. This will be the population used for all the pharmacokinetic displays.

9.3.1. Statistical Analyses

Details of the planned statistical reporting are described in the RAP.

9.3.2. Efficacy Analyses

This is not applicable.

9.3.3. Safety Analyses

All safety analyses will be performed on the All Participants Population. Safety data will be presented in tabular format and summarized descriptively according to GSK's Integrated Data Standards Library (IDSL. Further details will be given in the RAP.

9.4. Pharmacokinetic Analyses

Plasma linerixibat concentration-time data will be listed for each subject and summarised by treatment and planned sampling time. [^{14}C]-linerixibat and radioactivity concentrations in plasma will be reported similarly. Individual subject, mean and median plasma linerixibat, [^{14}C]-linerixibat, and total radioactivity concentration–time profiles will be plotted for each treatment on both a linear and semi-log scale.

Pharmacokinetic analysis will be performed by or under the direct auspices of Clinical Pharmacology Modelling & Simulation, GSK. Plasma linerixibat, [^{14}C]-linerixibat, and total radioactivity concentration–time data will be analysed by non-compartmental methods with WinNonlin Version 6.3 or above. Calculations will be based on the actual sampling times recorded during the study. From the plasma concentration-time data the following pharmacokinetic parameters will be determined, for linerixibat, [^{14}C]-linerixibat, and total radioactivity, as data permits: maximum observed plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration-time curve [AUC(0–t) and AUC(0-inf)], terminal phase rate constant (λ_z), and apparent terminal phase half-life ($t_{1/2}$) following oral and IV dosing. Additionally, volume of distribution at steady state (Vss), renal clearance (CLr), and total systemic clearance (CL) will be derived following IV dosing. These parameters will be summarised descriptively.

Calculation of bioavailability (F), Fraction of drug escaping first-pass hepatic clearance (Fh), and Fraction absorbed (Fa) for study 205895

Absolute oral bioavailability (F)	F = (AUCpo/Dosepo)/(AUCiv/Doseiv) Note: All AUC terms refer to AUC(0-inf)	Equation 1
Fraction of drug escaping first-pass hepatic clearance (Fh)	Fh = (1-ERh) Where ERh = Hepatic extraction ratio ERh = CLh / Qh Where, CLh = CLiv - CLrenal Hepatic blood flow (Qh) =87 L/h (Davies, 1993)	Equation 2a Equation 2b Equation 2c
Fraction of drug absorbed and fraction escaping gut clearance (Fa)	Fa = F/Fh	Equation 3

Derivation of the urine and fecal radioactivity parameters will be the responsibility, or under the direct auspices, of the BIB department within GSK. The following radioactivity parameters will be determined from the urine and fecal radiolabelled drug-related material (total radioactivity) data, and will be listed and summarised by treatment:

- Percentage excreted in urine (Fe%[urine]) within each collection period and cumulative urinary recovery and fraction excreted over the total collection period.
- Percentage excreted in feces (Fe%[fecal]) with each collection period and cumulative fecal recovery and fraction excreted over the total collection period and cumulatively over the collection period.
- Total excretion (sum of urine and fecal excretion), Fe% [total] will be calculated by collection interval for each subject.

The urine, fecal and total radioactivity parameters will be listed, summarised and plotted, as appropriate.

All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals, R&D. Production of the summaries, listings and figures of the plasma, urine and feces data will be performed under the direct auspices of Clinical Statistics, GSK.

Further details regarding the tables, figures and listings to be produced for the study report will be given in the RAP.

9.5. Metabolite profiling

The metabolic profiling/structural characterisation aspect of this work will be performed by GSK (or a GSK representative) in a separate nonclinical study and reported separately.

9.6. Interim Analyses

No interim analyses will be performed.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1. Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (e.g., advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

10.1.2. Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3. Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant and answer all questions regarding the study.

- Participants must be informed that their participation is voluntary. will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.

10.1.4. Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

10.1.5. Dissemination of Clinical Study Data

- Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.
- GSK will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study participants, as appropriate.
- The procedures and timing for public disclosure of the protocol and results summary and for development of a manuscript for publication for this study will be in accordance with GSK Policy.
- GSK intends to make anonymized participant-level data from this trial available to external researchers for scientific analyses or to conduct further research that can help advance medical science or improve patient care. This helps ensure the data provided by trial participants are used to maximum effect in the creation of knowledge and understanding.

10.1.6. Data Quality Assurance

All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (e.g., laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (e.g., risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The sponsor assumes accountability for actions delegated to other individuals (e.g., Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

10.1.7. Source Documents

- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Definition of what constitutes source data can be found in the SRM.

10.1.8. Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. The study site will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

10.1.9. Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.2. Appendix 2: Clinical Laboratory Tests

- The tests detailed in Table 4 will be performed by the local laboratory.
- The requirement for fasting before laboratory sample collection will be determined by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 5.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 4 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Haematology	Platelet Count RBC Count Hemoglobin Haematocrit	RBC Indices: MCV MCH Absolute and % Reticulocytes	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	Urea Creatinine Uric acid Fasting glucose Total cholesterol LDL cholesterol HDL cholesterol Triglycerides	Potassium Sodium Chloride Calcium Phosphate	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT) Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT) Alkaline phosphatase	Total and direct bilirubin Total Protein Albumin Globulin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase by dipstick • Microscopic examination (if leukocyte esterase, nitrites, blood or protein is abnormal)			
Screening only Tests	• Alcohol breath test, urine cotinine test • Carbon Monoxide (CO) breath test • Drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines) • Serology (HIV antibody, hepatitis B surface antigen [HBsAg], and hepatitis C virus antibody)			

NOTES :
[1] Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 7.1.1 and Appendix 8 (Section 10.8). All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE.

10.3. Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

10.3.1. Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study intervention.
Events <u>Meeting</u> the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.
Events <u>NOT</u> Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).

10.3.2. Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

A SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
Results in persistent disability/incapacity • The term disability means a substantial disruption of a person's ability to conduct normal life functions. • This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Is a congenital anomaly/birth defect
Other situations: • Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment

in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3. Recording and Follow-Up of AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

10.3.4. Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data collection tool.
- If the electronic system is unavailable, then the site will use the paper SAE data collection tool (see next section) in order to report the event within 24 hours.
- The site will enter the SAE data into the electronic system as soon as it becomes available.

- The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor or SAE coordinator by telephone.
- Contacts for SAE reporting can be found in the SRM.

SAE Reporting to GSK via Paper CRF

- Facsimile transmission of the SAE paper CRF is the preferred method to transmit this information to the medical monitor or the SAE coordinator.
- In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the investigator to complete and sign the SAE CRF pages within the designated reporting time frames.
- Contacts for SAE reporting can be found in the SRM.

10.4. Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

10.4.1. Contraception Guidance

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in (see Section 5.1):
    - Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
    - Agree to use a male condom when having penile-vaginal intercourse with a woman of childbearing potential
- Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration during the protocol-defined time frame.
- In addition, male participants must refrain from donating sperm for during the protocol-defined time frame.

10.4.2. Collection of Pregnancy Information:

For male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to male participants who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 24 hours of learning of the partner's pregnancy.
- The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK.
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

10.5. Appendix 5: Risk Assessment

Potential Risks of Clinical Significance, Relevant Data, and Mitigation Strategy

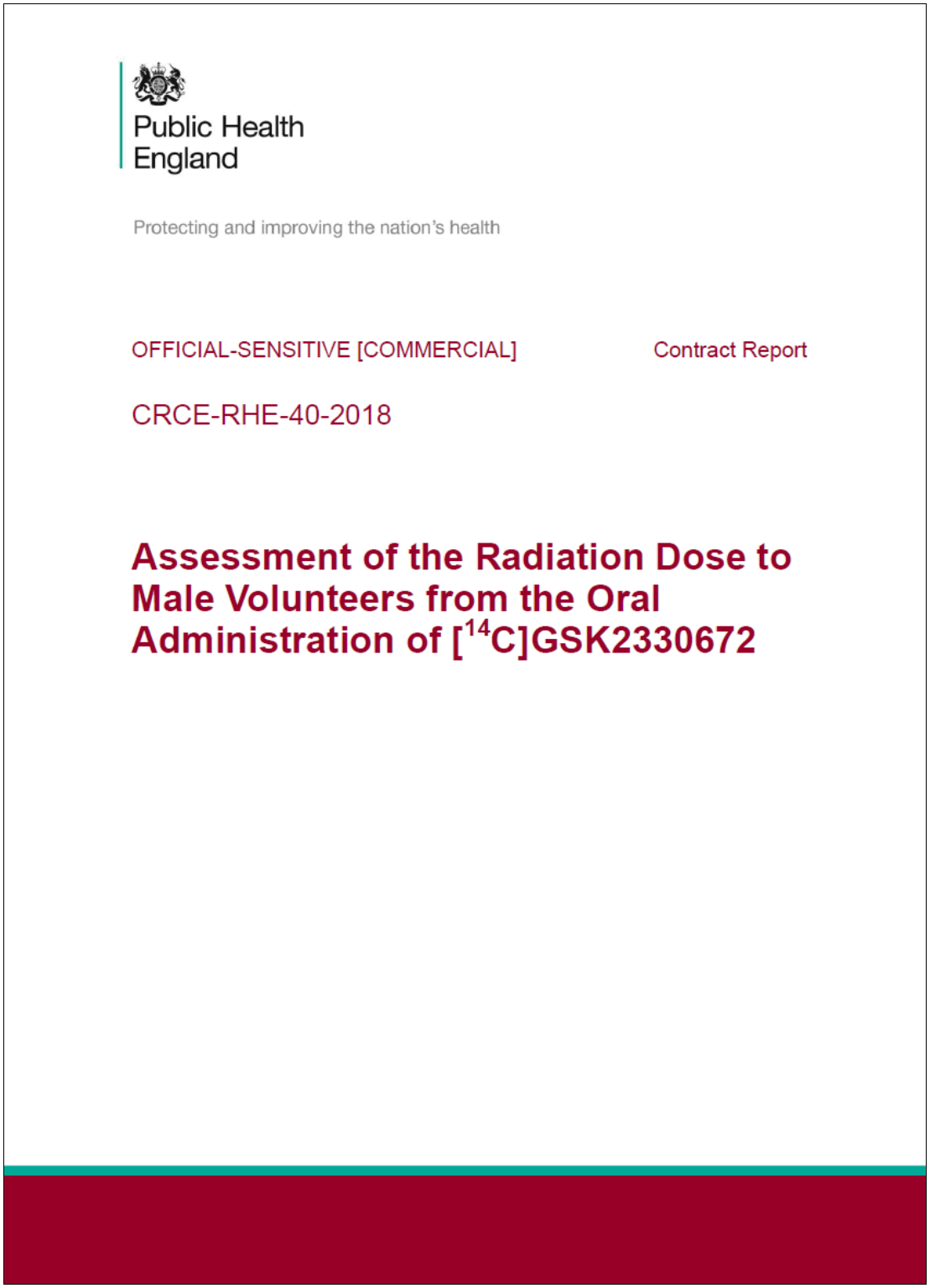
Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention: Linerixibat		
Systemic exposure to linerixibat greater than that previously tested in humans	The total systemic exposure of linerixibat from either treatment period (90 mg) is predicted to be below the systemic exposure from the highest dose of linerixibat tested in previous human studies (repeat oral doses of 180 mg). The 100 µg microtracer dose administered as a 3-h IV infusion is projected (based on allometry) to achieve a linerixibat Cmax <0.5ng/mL, which is 13-fold lower than the highest observed human Cmax following a 180 mg dose (Study 205808). Preclinical toxicology species provide safety cover for systemic exposure to linerixibat. The in vitro studies using hepatocytes from nonclinical species and humans show that there is generally no qualitative difference in metabolites formed. Preliminary non-radiolabelled studies detected no linerixibat metabolites in human plasma and urine following 90 mg BID dosing for two weeks (Study 200185).	Haematology and clinical chemistry, ECG, and vital signs will be monitored.
Gastrointestinal effects, including diarrhoea.	Animal studies including fecal alterations, e.g., nonformed, liquid (see IB, Section 4.5). AEs in humans including loose	Exclusion of participants with current episode, recent history, or chronic history of diarrhoea (see Section 5.2).

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	stools (see IB Section 5.3).	Only two doses of GSK23320672 separated by at least 13 days will be administered in this study.
Potential gallstones due to interruption of enterohepatic recirculation of bile acids.	Report of gallstone formation associated with genetic polymorphisms of the IBAT gene SLC10A2 in human studies (see IB, Section 5.3.1). A nonfatal SAE of acute cholecystitis leading to hospitalization for cholecystectomy and withdrawal from study intervention: Prior to dosing with metformin + linerixibat, the participant experienced diarrhoea, back pain, and WBC count above the reference range. The SAE was considered not attributable to study drug by the investigator (see IB Section 5.3.1).	Exclusion of participants with current symptomatic cholelithiasis or inflammatory gall bladder disease (see Section 5.2). Study subjects will also be informed that a possible risk of gallstones exists, but that studies in participants taking linerixibat have not shown an increase in reports of gallstones. Investigative staff will monitor participants for symptoms and signs of cholelithiasis. Participants will be informed of the signs and symptoms of cholelithiasis in the Informed Consent Form.
Radiolabelled Investigational Product: [^{14}C]-linerixibat		
Radioactivity exposure risk ([^{14}C]-linerixibat	The total effective dose associated with IV and oral administrations of [^{14}C]-linerixibat is 0.99 mSv. On this basis, the maximum administered activity would comply with the ICRP, 1992 recommendation of a 1 mSv maximum for Category IIa projects and well within the dose limits for members of the public, as per WHO Category II (further details are in Appendix 6, Table 2).. The total effective dose is also below the GlaxoSmithKline Global Safety Board level of radiation (10	The Period 1 dose is a microdose for which the associated radioactivity is less than the International Commission on Radiological Protection (ICRP, 1992) recommended threshold for clinical research projects with intermediate level of societal benefit. It is below the level identified as trivial risk by the ICRP, 1992) and below the maximum exposure threshold for the level of risk that is within variations of natural background radiation

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	mSv).	according to the World Health Organization (WHO, 1977). See Section 4.3 and Appendix 6 (Section 10.6). In Period 2, participants will be monitored for recovery of radioactivity (see Section 4.1.4).
Study Procedures		
Local extravasation of intravenous microdose at the infusion site	Linerixibat has not previously been administered by IV infusion in humans. In a single dose IV rat study, redness was observed around the injection site in a single rat: Male rats (n=4/group) were given single IV doses of linerixibat at 5, 15 or 30 mg/kg (slow bolus) and 30 or 60 mg/kg (3 or 4-hour infusion, respectively). All doses were well tolerated. One rat given 30 mg/kg by slow bolus injection had redness around the injection site. However, no injection site findings were reported in a repeat dose IV study where male rats (n=4/group) were given linerixibat IV at 2, 15 or 60 mg/kg/day once daily for 6 or 7 days. Linerixibat was also given to dogs as a single escalating IV dose at 5 or 15 mg/kg (slow bolus to 2 males and 1 female) or at 15, 30 or 60 mg/kg (4-hour infusion to 1 male and 2 females). A washout period of at least 3 days was allowed between initial and subsequent doses.	Participants will avoid moderate or strenuous physical activity during the infusion. Usual iv care will include flushing the iv before administration of the dose and monitoring for correct positioning of the iv. Study medication must be discontinued if the subject develops severe pain, redness, bleeding or swelling at the infusion site.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	No irritancy at site of injection was reported.	
String test for bile collection risk	Streaks of blood on the string due to local irritation have been infrequently noted. Gagging upon retrieval of the string can occur. On a few occasions, an entire string has been swallowed without ill effects and passes out from the body in the faeces.	The string will be securely taped in place (to the cheek of each individual) during the collection time to minimise risk of swallowing the entire string.

10.6. Appendix 6: An assessment of the radiation dose to male volunteers from the oral and IV administration of the study intervention



Public Health England

Protecting and improving the nation's health

OFFICIAL-SENSITIVE [COMMERCIAL] Contract Report

CRCE-RHE-40-2018

Assessment of the Radiation Dose to Male Volunteers from the Oral Administration of [^{14}C]GSK2330672

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Approval: October 2018
Publication: October 2018
File Reference Number B340/887

Contents

1. Introduction

GlaxoSmithKline have provided experimental data on the biokinetics of [^{14}C]GSK2330672 following its oral administration to pigmented and albino rats. The data have been used to determine the likely dose to male volunteers from a single administration of the radio-labelled compound. The assumption is made that the levels of uptake and retention by tissues will be the same in man as in the experimental animals. Committed equivalent doses to tissues and organs and committed effective dose (E(50)) have been calculated according to the 1990 Recommendations of the International Commission on Radiological Protection (ICRP)[1] as implemented in the Ionising Radiations Regulations 1999[2] in response to EU Council Directive 96/29/Euratom[3]. ICRP Publication 105[4] recommends the use of dose constraints, where the exposure of volunteers in biomedical research provides no direct benefit to them, to limit any inequity between individual risk and societal benefit and because there is no specific further protection in the form of a dose limit. The E(50) can be compared with the dose categories proposed for research projects involving human volunteers by the World Health Organization (WHO)[5] and ICRP[6].

2. Data used in calculations

Results were provided for the urinary and faecal excretion of ^{14}C following the oral administration of [^{14}C]GSK2330672 to albino rats. Expressed as percentages of total excretion, the values used for the calculations were 0.01% urinary and 99.9% faecal excretion.

Data were provided for the tissue distribution of ^{14}C after oral administration of [^{14}C]GSK2330672 to pigmented rats. Concentrations of radio-labelled compound retained in the tissues were expressed as nanogram equivalents per gram (ng eq/g) of tissue. These concentrations were converted to values for the percentage of administered activity retained in individual tissues or organs using standard organ weights[7]. Doses were calculated using values for 5 tissues at 3 time-points between 1 and 24 hours after administration.

3. Method of calculation

The initial step is the calculation of the number of transformations (U) in each source region from 1 Bq of administered drug. These values were calculated for the period for which data were supplied by the trapezoidal method of integration. Any fraction of the ^{14}C remaining in tissues or organs at the last

time point was assumed, conservatively, to be lost with a half-time of 100 days.

The values of U for the contents of the gut compartments, gall bladder and urinary bladder were calculated, as recommended by Dolphin and Eve[8], from the fraction of the administered activity passing through their contents and their mean residence time in each compartment. Calculations were made assuming that 100% of the faecally excreted activity was released into the gut in bile.

In order to calculate committed equivalent doses, $H_T(50)$ (Sv), to the target organs, the values of U are combined with a set of values known as Specific Effective Energies (SEEs)[9]. In short, the SEEs give the dose to each target region, T, per transformation in each source organ, S.

$$H_T(50) = \sum_S U(S,50) \times SEE(T,S)$$

The SEEs are calculated using data on absorbed fractions, (Φ) derived from a mathematical phantom[9], representing a reference adult male body, with additional data for the prostate taken from Stabin[10]. Absorbed fractions represent the fraction of energy emitted in each source that is absorbed in each target. In simple cases where S and T are the same, e.g. liver, the absorbed fraction for non-penetrating radiations is equal to one. The expression for SEE can be simply written

$$SEE(T,S) = \frac{\varepsilon}{m_T}$$

where ε is the mean energy of the emission (J) and m_T is the mass of the target organ (kg). The organ masses used for the calculations are those specified by the ICRP[11].

The committed effective dose, E(50), is the sum of the committed equivalent doses to individual tissues or organs, each weighted to allow for the relative contributions of tissues and organs to the total detriment; taking account of the probability of attributable fatal cancer, the weighted probability of attributable non-fatal cancer, the weighted probability of severe hereditary effects and the relative length of life lost[1]. Thus:

$$E(50) = \sum_T w_T \cdot H_T(50)$$

where $H_T(50)$ is the committed equivalent dose in tissue or organ T and w_T is the tissue weighting factor.

Weighting factors are specified for testes (0.2); colon, lung, red bone marrow and stomach (each 0.12); bladder, breast, liver, oesophagus and thyroid (each

0.05) and bone surfaces and skin (each 0.01)[1]. A complication is that the current dosimetric model of the gastrointestinal tract does not consider doses to the oesophagus, and divides the colon into upper and lower large intestine[12]. Until a revised model is available, doses to the oesophagus have been calculated using the thymus data (this is a standard dosimetric procedure justified for penetrating photon radiation on the basis of the proximity of the oesophagus and thymus). The dose to the colon is taken to be the mass weighted mean of the doses to the upper large intestine and lower large intestine. Doses from ^{14}C in transit through the gut were calculated separately from doses from activity retained in the gut wall. The mass weighted average of the dose to remainder tissues is given a total weighting factor of 0.05 unless any one tissue exceeds the highest equivalent dose to named tissues when it is attributed a weighting factor of 0.025; the weighting factor for the remainder becomes 0.025.

4. Results

The results of the calculation of committed effective dose, E(50), from the oral administration of [^{14}C]GSK2330672, based on the animal data supplied, are given in Table 1. The E(50) is the sum of the weighted equivalent doses to named tissues and the remainder tissues. The E(50) to a male volunteer following the oral administration of [^{14}C]GSK2330672 was calculated as 2.0×10^{-10} Sv Bq^{-1}. The dose to the colon contributed 95% of the E(50).

5. Conclusions

The E(50) value obtained for oral administration of [^{14}C]GSK2330672 to male volunteers was 2.0×10^{-10} Sv Bq^{-1} (Table 1). On this basis, the maximum administered activity that would comply with the WHO[5] recommendation of a 0.5 mSv maximum for Category 1 projects (see Table 2) would be 2.5 MBq (67.7 µCi). To comply with the ICRP[6] Category 1 limit of 0.1 mSv (see Table 2), the maximum activity would be 0.5 MBq (13.5 µCi).

6. Table 1

Estimated radiation doses to human tissues after oral administration of [^{14}C]GSK2330672 (Based on animal data)

Tissues	w_T	Equivalent Dose (Sv Bq^{-1})	Equivalent Dose x w_T (Sv Bq^{-1})	Contribution to effective dose (%)
Testes	0.2	0.00E+00	0.00E+00	0.00
Red bone marrow	0.12	0.00E+00	0.00E+00	0.00
Colon	0.12	1.60E-09	1.92E-10	95.05
Lungs	0.12	0.00E+00	0.00E+00	0.00
Stomach	0.12	7.60E-11	9.11E-12	4.52
Urinary Bladder	0.05	2.38E-13	1.19E-14	0.01
Breasts	0.05	0.00E+00	0.00E+00	0.00
Liver	0.05	1.63E-11	8.15E-13	0.40
Oesophagus	0.05	0.00E+00	0.00E+00	0.00
Thyroid	0.05	0.00E+00	0.00E+00	0.00
Skin	0.01	0.00E+00	0.00E+00	0.00
Bone surfaces	0.01	0.00E+00	0.00E+00	0.00
Remainder	0.05	7.98E-13	3.99E-14	0.02
Highest remainder tissue	0.00	0.00E+00	0.00E+00	0.00
Effective dose			**2.02E-10**	100.00
	Mass (g)		**Equivalent Dose x Mass**	
Adrenals	14	0.00E+00	0.00E+00	
Brain	1450	0.00E+00	0.00E+00	
Eyes (pigmented region)	1.5	0.00E+00	0.00E+00	
Heart	330	0.00E+00	0.00E+00	
Kidneys	310	0.00E+00	0.00E+00	
Muscle	29000	0.00E+00	0.00E+00	
Pancreas	140	0.00E+00	0.00E+00	
Pituitary	0.6	0.00E+00	0.00E+00	
Prostate	17	0.00E+00	0.00E+00	
Spleen	150	0.00E+00	0.00E+00	
Small intestine	650	1.82E-11	1.19E-08	
Gall bladder	10	1.38E-09	1.38E-08	
Thymus	25	0.00E+00	0.00E+00	

Category 1 Limits:	WHO 1977 <0.5 mSv	**2.48 MBq (67.7 µCi)**
	ICRP 1992 <0.1 mSv	**0.50 MBq (13.5 µCi)**

7. Table 2

Categories of risk and corresponding level of benefit for human exposure in biomedical research

WHO 1977

Category	Effective dose equivalent	Level of risk
I	Less than 0.5 mSv	Within variations of natural background
II	More than 0.5 mSv but less than 5 mSv	Within dose limits for members of the public
III	More than 5 mSv but less than 50 mSv	Within dose limits for persons occupationally exposed to radiation

ICRP 1992

Level of risk	Risk category	Corresponding effective dose range (adults) (mSv)	Level of societal benefit
Trivial	Category I ($\approx 10^{-6}$ or less)	<0.1	Minor
Minor to intermediate	Category II IIa ($\approx 10^{-5}$) IIb ($\approx 10^{-4}$)	0.1 - 1 1 – 10	Intermediate to moderate
Moderate	Category III ($\approx 10^{-3}$ or greater)	>10	Substantial

8. References

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10.7. Appendix 7 Genetics

USE/ANALYSIS OF DNA

- Genetic variation may impact a participant's response to study intervention, susceptibility, severity and progression of disease. Variable response to study intervention may be due to genetic determinants that impact drug absorption, distribution, metabolism, and excretion; mechanism of action of the drug; disease etiology; and/or molecular subtype of the disease being treated. Therefore, where local regulations and IRB/IEC allow, a blood sample will be collected for DNA analysis.
- DNA samples will be used for research related to linerixibat, pruritus associated with PBC, and related diseases. They may also be used to develop tests/assays (including diagnostic tests) related to linerixibat, drugs of this class, or PBC pruritus. Genetic research may consist of the analysis of one or more candidate genes or the analysis of genetic markers throughout the genomic analysis of the entire genome (as appropriate).
- DNA samples will be analyzed if it is hypothesized that this may help further understand the clinical data obtained from this study.
- DNA samples will be analyzed by using appropriate descriptive and/or statistical analysis methods. A detailed description of any planned analyses will be documented in a RAP prior to initiation of the analysis
- The samples may be analyzed as part of a multi-study assessment of genetic factors involved in the response to study intervention or study interventions of this class. The results of genetic analyses may be reported in the clinical study report or in a separate study summary.
- The sponsor will store the DNA samples in a secure storage space with adequate measures to protect confidentiality.
- The samples will be retained while research on linerixibat continues but no longer than 15 years after the last participant's last visit or shorter period as per local requirements.
- The samples will be retained while research on linerixibat, study interventions of this class, or PBC pruritus continues but no longer than 15 years after the last subject last visit or other period as per local requirements.

10.8. Appendix 8: Liver Safety: Required Actions and Follow-up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure participant safety and to evaluate liver event aetiology.

These protocol guidelines are in alignment with FDA premarketing clinical liver safety guidance.

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM174090.pdf

Table 5 Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="3">Liver Chemistry Stopping Criteria</th></tr>
<tr><td>ALT-absolute</td><td colspan="2">ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >1.5, Report as an SAE.
See additional Actions and Follow Up Assessments listed below</td></tr>
<tr><th colspan="3">Required Actions and Follow up Assessments</th></tr>
<tr><th>Actions</th><th colspan="2">Follow Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• Report the event to GSK within 24 h
• Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the participant until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 h
• Monitor participants twice weekly until liver</td><td colspan="2">• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend
• Obtain blood sample for pharmacokinetic (PK) analysis if requested by the Medical Monitor (determined on a case-by-case basis)[4]
• Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
• Fractionate bilirubin, if total bilirubin≥2xULN
• Obtain complete blood count with differential to assess eosinophilia
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form</td></tr>
</table>

chemistries resolve, stabilise or return to within baseline • A specialist or hepatology consultation is recommended **If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:** • Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 h** • Monitor participants weekly until liver chemistries resolve, stabilize or return to within baseline	• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications. • Record alcohol use on the liver event alcohol intake case report form **If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

[1] Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that participant if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.

[2] All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to participants receiving anticoagulants.

[3] Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody

[4] Record the date/time of the PK blood sample draw and the date/time of the last dose of study intervention. Sample handling and shipping instructions are in the SRM.

10.9. Appendix 9: Abbreviations and Trademarks

Abbreviations

AE	Adverse Event
ALT	Alanine aminotransferase
AMS	Accelerator Mass Spectrometry
AUC	Area under concentration-time curve
AUC(0–inf)	Area under the concentration-time curve from time zero (pre-dose) extrapolated to infinite time
AUC(0–t)	Area under the concentration-time curve from time zero (pre-dose) to last time of quantifiable concentration within a participant across all treatments
BIB	Bioanalysis, Immunogenicity and Biomarkers
BID	Bis In Die (Twice A Day)
BMI	Body mass index
BP	Blood Pressure
Bq	Becquerel
C_{max}	Maximum observed concentration
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations (US)
CRF	Case Report Form
CSR	Clinical Study Report
CV	Cardiovascular
DCSI	Development Core Safety Information
ECG	Electrocardiogram
F	Absolute bioavailability
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GI	Gastrointestinal
GSK	GlaxoSmithKline
h	Hour(s)
HBsAg	Hepatitis B Surface Antigen
Hep B	Hepatitis B
Hep C	Hepatitis C
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HMR	Hammersmith Medicines Research
IB	Investigator's Brochure
IBAT	Ileal bile acid transporter
ICF	Informed Consent Form
ICRP	International Commission on Radiological Protection

IDSL	Integrated Data Standards Library
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	Intravenous
IVIVT	In vitro / In vivo Translation - Investigative Safety & Drug Metabolism, GSK
LC	Liquid Chromatography
LC/MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LSC	Liquid Scintillation Counting
LFTs	Liver Function Tests
MBq	Megabecquerel
µCi	Micro Curie
µg	Microgram
µSv	Microsievert
mg	Milligrams
mL	Millilitre
MSDS	Material Safety Data Sheet
mSv	Millisievert
nCi	Nano Curie
NOAEL	No Observed Adverse Effect Level
PK	Pharmacokinetic(s)
QTcF	QT duration corrected for heart rate by Fridericia's formula
RAP	Reporting and Analysis Plan
SAE	Serious Adverse Event
SoA	Schedule of Activities
SRM	Study Reference Manual
t_{max}	Time of occurrence of C_{max}
t½	Terminal phase half-life
UK	United Kingdom
ULN	Upper Limit of Normal
WHO	World Health Organisation
λz	Lambda-z (Terminal Phase Rate Constant)

Trademark Information

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