CLINICAL STUDY PROTOCOL

An Open Label, Single-Dose, Single-Period Study Designed to Assess the Mass Balance Recovery, Metabolite Profile and Metabolite Identification of [^{14}C]-Rencofilstat in Healthy Male Subjects

Short Study Title: Single Dose ADME Study of [^{14}C]-Rencofilstat in Healthy Male Subjects

Quotient Sciences Study Number:	QSC204643
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EudraCT Number:	2022-003789-20
IRAS ID	1007072
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SPONSOR/QUOTIENT SCIENCES CONFIDENTIAL

Document History

Protocol Version Number	Classification	Date
Version 3.0	Non-Substantial Amendment	22 Mar 2023
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Version 1.0	Original Protocol	21 Dec 2022

Details of the amendments can be found in Appendix 3.

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3 Synopsis

Sponsor:	Drug Substance:	EudraCT No.:	IRAS ID:
Hepion Pharmaceuticals, Inc.	[^{14}C]-rencofilstat	2022-003789-20	1007072

Title of Study:

An Open Label, Single-Dose, Single-Period Study Designed to Assess the Mass Balance Recovery, Metabolite Profile and Metabolite Identification of [^{14}C]-Rencofilstat in Healthy Male Subjects.

Principal Investigator:

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Objectives and Endpoints:

Objectives	Endpoints
Primary To determine the mass balance recovery after a single oral dose of carbon-14 ([^{14}C])-rencofilstat	Mass balance recovery of total radioactivity (TR) in all excreta (urine and faeces): CumAe and Cum%Ae
To determine % parent drug unchanged versus metabolites	Parent to total radioactivity ratio for whole blood based on AUC
To perform metabolite profiling and structural identification from whole blood, urine and faecal samples[a]	Collection of whole blood, urine and faeces samples for metabolite profiling, structural identification, and quantification analysis
Secondary To determine the routes and rates of elimination of [^{14}C]-rencofilstat	Mass balance recovery of TR in urine and faeces separately: Ae, %Ae, CumAe and Cum%Ae by interval
To identify the chemical structure of each metabolite accounting for more than 10% of circulating TR or accounting for 10% or more of the dose in excreta	Identification of the chemical structure of each metabolite accounting for more than 10% by AUC of circulating TR or accounting for 10% or more of the dose in excreta
To evaluate the extent of distribution of TR into blood cells	Evaluation of whole blood:plasma concentration ratios for TR
To provide additional safety and tolerability information for rencofilstat	To provide additional safety and tolerability information for rencofilstat by assessing: incidence of adverse events (AEs), physical examinations and change from baseline for vital signs, electrocardiograms (ECGs), and laboratory safety tests
To further explore the oral pharmacokinetics (PK) of rencofilstat	PK parameters for rencofilstat in whole blood and TR in plasma and whole blood following a single oral dose, including but not limited to: Tlag, Tmax, Cmax, AUC(0-last), AUC(0-inf), AUC(0-24), Lambda-z, T1/2, CL/F, Vz/F, MRT(0-last) and MRT(0-inf) as applicable

[a] Metabolite profiling and identification will be reported separately from the clinical study report as a standalone document.

Methodology:

This is a single centre, open-label, non-randomised, single period, single dose study in healthy male subjects. It is planned to enrol a single cohort of 6 subjects.

Each subject will receive a single 225 mg oral dose of [^{14}C]-rencofilstat self-microemulsifying drug delivery system oral emulsion (not more than [NMT] 6.4 MBq), in the fasted state.

Study Design:

Subjects will undergo preliminary screening procedures for the study at the screening visit (Day -28 to Day -2). Subjects will be admitted in the evening on the day before dosing (Day -1).

Whole blood, plasma, urine and faeces samples will be collected at regular intervals for PK analysis, TR analysis, metabolite profiling, mass balance and safety as applicable, from pre-dose to discharge from the clinical unit. Urine and faeces samples may be collected at return visits or home visits if mass balance criteria has not been met by a subject.

Subjects will be dosed on the morning of Day 1 following an overnight fast (minimum 8 h) and will remain resident in the clinical unit until up to 504 h after dosing (up to Day 22).

It is planned that subjects will be released as a group when all subjects have achieved a mass balance cumulative recovery of >90% or if <1% of the dose administered has been collected in urine and faeces within two separate, consecutive 24 h periods. This may result in the subjects being discharged as a group prior to completion of the planned residency period.

If the mass balance criteria are achieved during the planned residency period, collection of all samples (blood, urine and faeces) will be stopped once the current 24 h collection period is complete, and subjects will undergo discharge assessments.

If the mass balance criteria have not been met by all subjects on the morning of Day 22, the residency period for the subjects not achieving the mass balance criteria may be extended up to a maximum of 96 h (Day 26; 600 h post-dose). Only urine and/or faecal samples will be collected during the extended residency period.

If the mass balance criteria have not been met by all subjects on the morning of Day 26, subjects not achieving the release criteria may be required to make up to 2 return visits (each of 24 h duration) at (nominally) Day 28 (+2 days) and Day 35 (±2 days) for additional collection of excreta samples (urine and/or faecal samples).

If the mass balance criteria have not been met following the return visits, then home collections of urine and/or faeces may be requested at the discretion of the investigator for individual subjects.

Number of Subjects Planned:

It is planned to enrol 6 healthy male subjects to ensure data in a minimum of 4 subjects. No replacement subjects are planned for this study.

Duration of Study:

Subjects will receive 1 single administration on a single occasion. The estimated time from screening until the end of the study is approximately up to 8 weeks (9 weeks if return visits are required).

Main Inclusion Criteria:

Healthy males aged 30 to 65 years inclusive at the time of signing informed consent.

Body mass index of 18.0 to 35.0 kg/m^2 as measured at screening.

Investigational Medicinal Product, Dose and Mode of Administration:

The following IMP will be used in this clinical study.

IMP Name	Dose	Route of Administration
[^{14}C]CRV431 SMEDDS Concentrate for Oral Emulsion, 225 mg (NMT 6.4 MBq)	225 mg	Oral administration, Fasted

^{14}C: carbon-14, MBq: megabecquerel, NMT: not more than

Immediately after administration of the oral solution, the dosing vessel will be rinsed with water and subjects will consume the rinse solution. Subjects will then consume further water to a total volume of 240 mL (including the dosing volume and volume used to rinse the dosing vessel).

Mass Balance Assessments:

Mass balance of TR in urine and faeces (and emesis, if applicable) will be calculated by Quotient Sciences for the parameters below.

Parameter	Definition
Ae(urine)	amount of TR excreted in urine
%Ae(urine)	amount of TR excreted in urine expressed as a percentage of the radioactive dose administered
CumAe(urine)	cumulative amount of TR excreted in urine
Cum%Ae(urine)	cumulative amount of TR excreted in urine expressed as a percentage of the radioactive dose administered
Ae(faeces)	amount of TR eliminated in faeces
%Ae(faeces)	amount of TR eliminated in faeces expressed as a percentage of the radioactive dose administered
CumAe(faeces)	cumulative amount of TR eliminated in faeces
Cum%Ae(faeces)	cumulative amount of TR eliminated in faeces expressed as a percentage of the radioactive dose administered
Ae(total)	amount of TR excreted in urine and faeces combined
%Ae(total)	amount of TR excreted in urine and faeces combined expressed as a percentage of the radioactive dose administered
CumAe(total)	cumulative amount of TR excreted in urine and faeces combined
Cum%Ae(total)	cumulative amount of TR excreted in urine and faeces combined expressed as a percentage of the radioactive dose administered

Pharmacokinetic and Total Radioactivity Assessments:

Whole blood concentration-time data for rencofilstat will be analysed by Quotient Sciences using Phoenix WinNonlin v8.3 or a more recent version (Certara USA, Inc., USA) and appropriate non-compartmental techniques to obtain estimates of the following PK parameters, where possible and appropriate.

Parameter	Definition
Tlag	Time prior to the first measurable concentration
Tmax	Time of maximum observed concentration
Cmax	Maximum observed concentration
AUC(0-24)	Area under the curve from time 0 to 24 h post-dose
AUC(0-last)	Area under the curve from time 0 to the time of last measurable concentration
AUC(0-inf)	Area under the curve from time 0 extrapolated to infinity
AUCextrap	Area under the curve from time of the last measurable concentration to infinity as a percentage of the area under the curve extrapolated to infinity
AUC ratio	Parent to total radioactivity ratio for whole blood based on AUC
T1/2	Terminal elimination half-life
Lambda-z	First order rate constant associated with the terminal (log-linear) portion of the curve
CL/F	Total body clearance calculated after a single extravascular administration where F (fraction of dose bioavailable) is unknown
Vz/F	Apparent volume of distribution based on the terminal phase calculated using AUC(0-inf) after a single extravascular administration where F (fraction of dose bioavailable) is unknown
MRT(0-last)	Mean residence time from time 0 to time of the last measurable concentration

Parameter	Definition
MRT(0-inf)	Mean residence time from time 0 extrapolated to infinity

In addition, whole blood-to-plasma TR concentration ratios will be calculated.

Metabolite Profiling and Identification Assessment:

Metabolite profiling of whole blood, urine and faeces will be performed using liquid chromatography-radio-detection with subsequent mass spectrometry where appropriate. Identification of the chemical structure of each metabolite accounting for greater than 10% of circulating radioactivity in whole blood ("AUC pool") and accounting for greater than 10% of the dose in the urine and faeces (from urine pools and faeces homogenate pools) will be performed. These aspects will be reported separately from the clinical study report as a standalone document.

Safety Assessments:

The safety assessments to be conducted are:

- AE monitoring
- Single 12-lead ECGs
- Vital signs
- Clinical laboratory tests (clinical chemistry, haematology and urinalysis)
- Physical examinations

Statistical Methodology:

No formal statistical analysis will be performed for this study. Descriptive statistics (eg mean, median, standard deviation, minimum, maximum and number of subjects with an observation [n]) are considered adequate for a study of this type. Additional statistics will be provided for PK-related data, including coefficient of variation (CV%), geometric mean and geometric CV%.

Sample Size and Power:

The study is exploratory and no formal sample size calculation has been made. Based on experience from previous studies of a similar design, a total of 6 subjects are to be enrolled and a minimum of 4 evaluable subjects are considered sufficient. An evaluable subject is defined as a subject who has provided mass balance and PK samples for up to 21 days after drug administration or have demonstrated >90% mass balance recovery or have <1% of the administered dose eliminated in excreta for two consecutive days, whichever is sooner.

4 List of Abbreviations

Abbreviation	Definition
^{14}C	carbon-14
ADME	absorption, metabolism, distribution and elimination
AE	adverse event
ALT	alanine aminotransferase
ARSAC	Administration of Radioactive Substances Advisory Committee
BCRP	breast cancer resistance protein
BMI	body mass index
CLcr	creatinine clearance
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	Coronavirus Disease 2019
CsA	cyclosporine A
CSPM	Clinical Sample Processing Manual
CV%	coefficient of variation
CYP450	cytochrome P450
Cyp A	cyclophilin A
Cyp B	cyclophilin B
Cyp D	cyclophilin D
DDI	drug-drug interaction
DMP	data management plan
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
GCP	good clinical practice
GLP	good laboratory practice
HBsAg	hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HCV Ab	hepatitis C virus antibody
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IMP	investigational medicinal product

ISF	Investigator Site File
MAD	multiple ascending dose
MATE	Multidrug and toxin extrusion protein
MHRA	Medicines and Healthcare products Regulatory Agency
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic Steatohepatitis
NMT	not more than
NOAEL	no-observed-adverse-effect-level
OAT	(human) organic ion transporter
PgP	P-glycoprotein
PIS	Participant Information Sheet
PK	pharmacokinetic(s)
QA	quality assurance
QD	once daily
QTcF	Corrected QT interval by Fridericia's formula
RAP	Reporting and Analysis Plan
SAD	single ascending dose
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SC	subcutaneous
SMEDDS	self-microemulsifying drug delivery system
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TDF	tenofovir disoproxil fumarate
TK	toxicokinetic
TR	total radioactivity
TV	tidal volume

The mass balance and pharmacokinetic definitions used in this study are presented in Section 15.3.1 and Section 15.3.2.

5 Background Information

5.1 Introduction

Rencofilstat, previously known as CRV431, is a non-immunosuppressive cyclosporine A (CsA) analogue being developed by Hepion Pharmaceuticals, Inc. for the treatment of fibrotic diseases, including Non-alcoholic Steatohepatitis (NASH) and Hepatocellular Carcinoma (HCC).

The molecular action of CRV431 is binding and inhibition of cellular enzymes called cyclophilins. Cyclophilins are peptidyl prolyl isomerases that catalyse changes in the isomeric conformation (cis and trans) of peptide bonds between prolines and adjacent amino acids. Cyclophilins are involved in diverse cellular process, including intracellular signalling and inflammation (Cyclophilin A [Cyp A]), protein folding and secretion (Cyclophilin B [Cyp B]), and regulation of mitochondrial permeability transition pores (Cyclophilin D [Cyp D]), which protect cells in response to injury.

Cyclophilins were first characterized through the discovery that cyclosporine A (CsA), a drug used for prevention of organ graft rejection, exerts its immunosuppressive activity by a two-step mechanism involving inhibition of Cyp A and calcineurin [1],[2],[3],[4]. Researchers have chemically modified CsA to diminish its calcineurin binding and thereby reduce its immunosuppressive properties while retaining its binding and inhibition of cyclophilins. This class of compounds is known as “non-immunosuppressive CsA analogues,” and includes rencofilstat. The safety and efficacy of this class of drug has been demonstrated by the partial clinical development of alisporivir wherein the compound was administered to over 2000 subjects [5],[6],[7].

Rencofilstat displays the basic functional characteristics of the family – high potency cyclophilin inhibition but low immunosuppressive activity – but it also possesses distinct properties that distinguish it from other compounds in the class such as increased solubility and less interaction with membrane transporters. The functional distinctiveness of rencofilstat derives in part from its unique modifications at amino acids 1 and 3, in comparison to most other non-immunosuppressive CsA analogues which are modified at amino acid positions 3 and 4.

Non-alcoholic fatty liver disease (NAFLD) and its more severe form, NASH are significant health burdens with prevalence increasing throughout the world. NAFLD is characterized by fat accumulation in the liver and often is associated with obesity, insulin resistance, dyslipidaemia and other aspects of metabolic syndrome. NASH shares these characteristics but additionally is characterized by liver inflammation and fibrosis. Extensive fibrosis in the absence of compensatory fibrolysis leads to scarring and cirrhosis and an increased likelihood of developing complications such as HCC, with an annual incidence of 0.5% to 2.6% [8]. HCC represents more than 80% of the primary liver cancers.

Worldwide NAFLD prevalence is 25% with the highest rates in the Middle East and South America. NASH affects between 1.5% and 6.5% of the global population. In the US, NAFLD prevalence is projected to increase from 83 million people in 2015 to 101 million people in 2030, with 33% of the population over the age of 15 impacted. The incidence of NASH and HCC are expected to similarly increase resulting in an excess 800,000 liver deaths during this period [9]. There are currently no US Food and Drug Administration (FDA) or UK Regulatory authority approved drugs for NASH, and metabolism-regulating drugs are largely not efficacious. Most drugs currently under development target metabolic disease rather than fibrosis. Multiple cyclophilin isoforms potentially contribute

to NASH pathophysiology, including Cyp A, Cyp B, and Cyp D, by participating in energy and lipid metabolic activities, regulation of hepatocyte death, inflammation, and fibrosis [10].

Reduction of liver steatosis, hepatocyte death, inflammation, and especially fibrosis are the primary outcomes being sought in NASH clinical trials. Achieving these endpoints is expected to alleviate the risks of advancing to compensated cirrhosis, decompensated cirrhosis, HCC, and liver failure [10].

5.2 Investigational Medicinal Product

The investigational medicinal product (IMP) that will be used in this clinical study is presented in Table 1.

Table 1 Investigational Medicinal Product

IMP Name	Dose	Route of Administration
[^{14}C]CRV431 SMEDDS Concentrate for Oral Emulsion, 225 mg (NMT 6.4 MBq)	225 mg	Oral administration, Fasted

^{14}C: carbon-14, MBq: megabecquerel, NMT: not more than, SMEDDS: self-microemulsifying drug delivery system

The carbon-14 ([^{14}C])-rencofilstat (also referred to as [^{14}C]CRV431) self-microemulsifying drug delivery system (SMEDDs) oral emulsion is an un-licensed medicinal product for use only in the proposed clinical trial.

Only subjects enrolled in the study may receive study treatment and only authorised site staff may supply or administer study treatment. All study treatments will be stored in a secure, environmentally-controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the investigator and authorised site staff.

[^{14}C]-rencofilstat will be manufactured in accordance with Good Manufacturing Practice (GMP) by Quotient Sciences. Where Quotient Sciences (hereafter referred to as Quotient) is manufacturing the final [^{14}C]-rencofilstat SMEDDs oral emulsion, suitability of the manufacturing process will be documented in a Pharmaceutical Development and Control Strategy Report.

IMP will be reconciled and destroyed in accordance with the study-specific quality agreement and technical addendum.

5.3 Previous Study Findings

Full details of previous study findings can be found in the Investigator's Brochure (IB) [11]. A summary of the non-clinical and clinical findings is provided below.

5.3.1 Non-clinical Findings

Non-clinical Pharmacology

Rencofilstat has been shown to bind specifically and with high potency to several human cyclophilin isoforms including Cyp A, Cyp B, Cyp D, and cyclophilin G. Across different species, organ types, and experimental systems, rencofilstat treatment resulted in therapeutic effects. Notably, rencofilstat decreased markers of inflammation and fibrosis in vitro, such as collagen and fibronectin secretion, and decreased liver fibrosis by 37% to 82% in disease models.

Anti-neoplastic properties of rencofilstat were examined both *in vitro* and *in vivo*. Rencofilstat alone or in combination with other anti-cancer agents decreased growth of tumours and reduced tumour burden in mouse models.

Rencofilstat demonstrated no indication of cytotoxicity, mitochondrial toxicity, immunosuppression, or off-target safety findings, consistent with long-term toxicology studies in rat and monkey that showed no rencofilstat-induced toxicity.

Non-clinical Pharmacokinetics and Metabolism

The PK characteristics of rencofilstat including drug absorption, distribution, and metabolism were determined in vitro and in rat and monkey. Seven-day, 30-day, 26-week, and 39-week TK studies were conducted in rat and monkey.

Whole blood is the preferred matrix as rencofilstat partitions primarily in red blood cells at concentrations ≤ 2 µM, and predominantly in the plasma fraction at 10 µM. Rencofilstat exhibited >96% plasma protein binding and is stable in rat, monkey and human whole blood and plasma for at least 8 h.

Rencofilstat was predominantly metabolized by cytochrome P450 (CYP450) 3A4 into at least 12 distinct metabolites which is consistent with the reported metabolism of other cyclosporine analogues. Rencofilstat was not a significant inhibitor of any CYP450s and was not found to be an enzyme inducer.

The pharmacokinetics (PK) of rencofilstat in rats and monkeys showed an increase in exposure with dose but appeared to reach a saturation point at the higher doses with a Tmax ranging from 1 to 8 h.

Non-clinical Toxicology

In both acute (30-day) and chronic (6- and 9-month) toxicology studies in rats and monkeys, rencofilstat was well tolerated and did not result in notable changes in weight, appetence, or clinical signs and symptoms.

In rat, multiples of human exposure were up to 4.9-fold at the clinical dose of 75 mg and up to 3.4-fold relative to the 225 mg dose. In the monkey, multiples of human exposure were up to 5.4-fold at the clinical dose of 75 mg and 3.7-fold relative to the 225 mg dose.

Genotoxicity studies included in vitro bacterial reverse mutation assay and mammalian chromosomal aberration test, and in vivo mammalian erythrocyte micronucleus test (conducted as part of the 30-day rat toxicity study). No genotoxicity findings were observed.

In single- and repeat-dose toxicity studies in rats and monkeys, no mortality, clinical signs, changes in body weight, or genotoxicity were observed throughout the studies. The NOAEL for both rat and monkey was considered to be the highest dose level tested or the highest dose before plateau of exposure.

5.3.2 Clinical Findings

Rencofilstat PK parameters, safety, and efficacy in humans was investigated in three Phase 1 clinical studies and one Phase 2 clinical study. To date there have been no deaths or serious adverse events (SAEs). Most TEAEs were mild or moderate in severity and occurred at comparable rates between placebo and rencofilstat. There are two ongoing clinical studies in NASH subjects.

Brief Safety Summary of Completed Clinical Studies

During the first in human study of rencofilstat in healthy volunteers (CRV431-101) there was no trend observed for adverse event (AE) reporting in relation to the rencofilstat dose. There were also no remarkable observations in the remaining safety assessments for vital signs, 12-lead ECGs, or safety laboratory measurements in this study.

The result of CRV431-103 study in healthy volunteers indicated that rencofilstat does not alter the PK of midazolam or its metabolite, 1-hydroxymethyl midazolam. Midazolam and ketoconazole did alter the PK of rencofilstat, resulting in approximately 2-fold and 4-fold higher exposures, respectively. One subject was discontinued from study participation due to an AE of elevated alanine aminotransferase (ALT)/AST who was later determined to have infection with Coronavirus Disease 2019 (COVID-19).

Administration of rencofilstat with a high fat meal in study CRV431-104 led to increases in Cmax, AUC(0-24), and AUC(0-inf) fed to fasted geometric mean ratios of 102.2%, 114.5% and 132.9%, respectively. The upper bounds of the 90% confidence intervals extended beyond the 80-125% range for AUC(0-last) and AUC(0-inf), All AUC geometric mean ratios were outside of the 80–125% range, suggesting a high fat meal can increase the extent of rencofilstat exposure. Tmax increased from 1.5 to 1.8 h in the fasted and high fat groups, respectively, suggesting slightly delayed absorption. Mean half-life was approximately 22 h longer for subjects administered rencofilstat with food compared to subjects administered rencofilstat under fasted conditions, although inter-subject variability was considerably higher for the fed cohort.

During CRV431-201 study, a multiple dose study in patients with NASH, two subjects discontinued the study treatment due to an AE; one subject experienced palpitations leading to discontinuation on study day 3 and another withdrew due to myalgia, migraine, and pyrexia on study day 7. A total of 21 (44.7%) subjects experienced 36 AEs, with most (69.4%) being unrelated to study treatment. A majority (97.2%) were mild to moderate in severity. One AE, constipation, was reported as severe (Grade 3), and there were no deaths or SAEs reported in the study. There were no AEs related to change in laboratory values, including liver biochemical markers, reported except for one subject with hypercholesterolaemia and one subject with a urinary tract infection.

Overall, rencofilstat was deemed safe and well tolerated at all combinations tested to date in both healthy and NASH subjects.

Summary of Completed and Ongoing Clinical Studies to Date

Study ID Phase	Population	Study Title	Study design	Dosing regimen
Completed Clinical Studies				
CRV431-101 Phase 1	Healthy Subjects	A Randomized, Partially Blinded, Placebo-controlled, Ascending Sequential Dose Groups, Single Dose Study of the Safety, Tolerability and Pharmacokinetics of CRV431, Alone and in Combination with Tenofovir Disoproxil Fumarate and in Multiple Ascending Sequentially Dosed Healthy Subjects	Randomised, subject-blinded, placebo controlled ascending SAD and MAD	Part 1 (SAD): 75, 225, 375, or 525 mg PO Part 2 (DDI): 375 mg rencofilstat PO, 300 mg TDF PO, or combination Part 3 (MAD): 75, 150, 225, 300 or 375 mg PO QD, 28 days
CRV431-103 Phase 1	Healthy Subjects	A Single Center, Open-Label, Phase 1 DDI Study to Evaluate the Pharmacokinetics of CRV431 in Normal Healthy Volunteer Subjects	Randomised, open label, Phase 1, single-centre DDI study	75 mg rencofilstat PO alone or in combination with 400 mg ketoconazole PO or 2 mg midazolam IV
CRV431-104 Phase 1	Healthy Subjects	A Single Center, Open-Label, Phase 1 Study to Evaluate the Pharmacokinetics of CRV431 Under Fasted and Fed States in Normal Healthy Volunteer Subjects	Open label, single dose	225 mg PO
CRV431-201 Phase 2a	Presumed F2/F3 NASH Subjects	AMBITION: A Phase 2a, Multi-Center, Single-Blind, Placebo-Controlled Study to Evaluate the Safety and Tolerability of CRV431 Dosed Once Daily in NASH Induced F2 and F3 Subjects	Multi-centre, single blind, placebo controlled	75 mg or 225 mg PO QD, 28 days
Ongoing Clinical Studies				
CRV431-207 Phase 2b	NASH Subjects	ASCEND-NASH: A Phase 2B, Randomized, Multi-center, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Rencofilstat in Adult Subjects with Nonalcoholic Steatohepatitis and Advanced Liver Fibrosis	Multi-centre, double blind, placebo controlled	75 mg, 150 mg and 225 mg plus placebo, QD for 365 days PO
CRV431-210 Phase 2	NASH Subjects	ALTITUDE-NASH: A Phase 2, Randomized, Multi-center, Open-label Study to Evaluate the Safety and Efficacy of Rencofilstat in Adult Subjects with Nonalcoholic Steatohepatitis Stage 3 Fibrosis	Multi-centre, open label	75 mg, 150 mg and 225 mg QD for 120 days PO

DDI: drug-drug interaction, MAD: Multiple Ascending Dose, SAD: Single Ascending Dose, TDF: tenofovir disoproxil fumarate

Pharmacokinetics Summary of Single Ascending Doses in Healthy Volunteers

PK analyses have been performed in all of the completed clinical trials to date. Rencofilstat PK have been consistent and repeatable in both healthy volunteer subjects

and presumed F2/F3 NASH subjects. As the current study is a single dose study, only single ascending dose (SAD) PK parameters are summarised here.

In the CRV431-101 SAD part of the study, healthy volunteers received single doses of rencofilstat at 75, 225, 375, and 525 mg, or vehicle (liquid formulation), and PK was assessed for 6 days post-dose. Results demonstrated a plateauing of the apparent rate (Cmax) and extent (AUC) of rencofilstat exposure at dose levels above 225 mg. This was consistent with PK observations in rat and monkey that showed a plateau between middle and high dosing levels.

Single dose PK of rencofilstat approximated first-order behaviour from 75 mg to 225 mg with decreasing exposure after 375 mg. Dose proportionality was initially greater than 1:1 but decreased with the plateauing of exposure (Table 2).

Table 2 Summary of Whole Blood Rencofilstat Pharmacokinetic Parameters Following Single Oral Doses (SAD, PK Population)

PK Parameters	Rencofilstat 75 mg (N=6)	Rencofilstat 225 mg (N=6)	Rencofilstat 375 mg (N=6)	Rencofilstat 525 mg (N=6)
AUC(0-24) (ng.hr/mL)	6048 ± 24.9	17720 ± 27.8	15670 ± 29.3	18170 ± 18.2
AUC(0-last) (ng.hr/mL)	16620 ± 18.7	53530 ± 32.4	50640 ± 34.9	62340 ± 24.2
AUC(0-inf) (ng.hr/mL)	20550 ± 18.1	80030 ± 36.2	79980 ± 50.2	92870 ± 32.1
Cmax (ng/mL)	320.5 (32.0)	1354 (15.5)	1204 (58.3)	1640 (15.0)
Tmax (hr)	2.99 (2.00, 10.00)	1.00 (1.00, 2.00)	1.00 (1.00, 4.00)	1.00 (1.00, 100)
T1/2 (hr)	73.6 ± 15.2	98.14 ± 17.96	111.27 ± 37.25	94.05 ± 17.87
CL/F (L/hr)	3.7 ± 0.7	3.0 ± 0.9	5.2 ± 2.7	5.9 ± 1.6
Vz/F (L)	392.6 ± 115.50	412.7 ± 144.0	753.7 ± 244.7	765.7 ± 136.9

Cmax presented as geometric mean (geometric CV%). Tmax presented as median (minimum, maximum). Other parameters are presented as arithmetic mean ± SD.

Safety Summary of Single Ascending Doses in Healthy Volunteers

In the CRV431-101 SAD part of the study there were no deaths, SAEs, or subject discontinuations due to AEs in Part 1 of the study. Overall, a total of 20 AEs were experienced by 14 (47%) subjects following active treatment, with 3 (50%) subjects reporting 3 events following rencofilstat 75 mg, 1 (17%) subject reporting 5 events following rencofilstat 225 mg, 3 (50%) subjects reporting 3 events following rencofilstat 375 mg, 3 (50%) subjects reporting 5 events following rencofilstat 525 mg, 4 (67%) subjects reporting 4 events following rencofilstat 375 mg ingested immediately following dilution, and 6 (60%) subjects reported 15 events following placebo.

Mild constipation was the most commonly reported event, reported a total of 3 times by 3 (10%) active-treatment subjects in Part 1 of the study, with 1 subject each reporting 1 event following rencofilstat 375 mg, rencofilstat 525 mg, and rencofilstat 375 mg ingested immediately following dilution. The investigator considered all events to be possibly related to the study treatment. Each of the remaining AE terms were reported in ≤ 2 subjects (≤ 7%).

6 Rationale

6.1 Study Rationale

This study aims to understand the absorption, distribution, metabolism and elimination (ADME) of rencofilstat in humans further through the assessment of the mass balance recovery after a single oral dose of [^{14}C]-rencofilstat. The routes and rates of elimination of [^{14}C]-rencofilstat will be determined and whole blood, urine and faecal samples will be used for metabolic profiling and structural identification.

This is a single dose mass balance study of a single formulation, the study will be open-label and no randomisation is required.

6.2 Dose Rationale

The Phase I clinical study found that rencofilstat was safe and well tolerated at single doses from 75 mg to 525 mg and at multiple doses of 75 mg to 375 mg, administered daily for 28 days.

Systemic exposure of rencofilstat as measured by Cmax and AUC are approximately 10 to 100-fold above the drug's IC50 for inhibition of cyclophilins. Thus, the doses tested from the 75 mg dose upwards achieve systemic concentrations necessary to adequately engage the drug receptors at the site of action. Doses of 75 mg and 225 mg per day for 28 days were studied in the Phase 2 study in patients with NASH. A dose of 225 mg has been chosen for this study as in previous clinical studies, rencofilstat systemic exposure has been found to plateau at doses equal to and higher than 225 mg. It is expected that 225 mg will be the most efficacious dose and is currently the highest dose being evaluated in the Phase II clinical trials.

The dose of radioactivity has been determined following review of human dosimetry calculations provided by UK Health Security Agency. The associated radiation exposure will fall within International Commission on Radiological Protection (ICRP) (1992) Guidelines for Category IIb studies (1 to 10 mSv). The dose of radioactivity will be no more than 6.4 MBq.

To ensure that the [^{14}C] drug product does not exceed the limit for radioactive dose approved by the Administration of Radioactive Substances Advisory Committee (ARSAC), the target specific activity of the drug substance will be set at 90% of 90% of the threshold radioactive dosing limit. This will allow for tolerances in the manufacturing processes for both drug substance and drug product thereby providing continued assurance for compliance with the ARSAC-approved limit for drug product radioactivity dose.

6.3 Population Rationale

As this is a Phase I study assessing the ADME, PK and safety of rencofilstat, the most relevant population is healthy volunteers. Subjects who are non-smokers without a history of alcohol or drug abuse or regular co-medication are proposed to avoid interaction on drug metabolism and to avoid non-compliance.

Data is not currently available from fertility and teratology studies with rencofilstat and subjects will be exposed to radiation; therefore, female subjects will not be enrolled in this study.

We acknowledge the ARSAC Notes for Guidance recommend that wherever possible, healthy subjects selected for research projects should be aged over 50 years [12]. However, the current study is designed to generate data for supporting the investigation of the human ADME of a drug, as well as generating samples for metabolite profiling and structural identification.

There are two main reasons for generating these data within a clinical development programme. The first is to provide human metabolite data that can be used to interpret the metabolism profiles seen in the preclinical species employed in the longer-term toxicity studies, to ensure that there is adequate toxicology coverage for the safe development of the drug in patients. The second is to provide data to understand how the drug is processed in physiologically normal subjects, because understanding the routes of metabolism and elimination in a healthy population generates the appropriate data to guide the clinical pharmacology package required to fulfil the regulatory requirements of a New Drug Application.

In order to address these two main aims of an ADME study, investigation of the drug under development is required in a population with normal physiological function, as it is recognised that certain physiological processes eg renal function, deteriorate with age and therefore it is preferable to use as healthy a population as possible, to mitigate against factors which may make interpretation of the data difficult. Also, healthy subjects as a trial population are ideal since they have a relatively stable physiological, biochemical and hormonal status, which removes any disease-related variations and variations due to concomitant medications. Therefore, given the aims of this ADME study our target age range for this study will be 30 to 65 years.

In addition, the initial target indication of this drug is NASH and, therefore, the target age range for the indication is included in the proposed age range for the study.

6.4 Risks and Benefits

6.4.1 Risks Associated with Rencofilstat Administration

Rencofilstat, previously known as CRV431, has been studied in three Phase I studies in healthy volunteers (CRV431-101, CRV431-103, and CRV431-104) as well as in a Phase 2a study in NASH patients (CRV431-201). CRV431-101 was a first in human study of rencofilstat comprised of 1) a SAD study; 2) a DDI study between rencofilstat and TDF; and 3) a 28-day MAD study. There were no deaths or SAEs in any parts of the study.

In Part 1, 20 AEs were experienced by 14 (47%) subjects following active treatment. The investigator assessed 18 events to be Grade 1, and 2 events to be Grade 2 in severity; 9 events were possibly related to study treatment, and 11 events were assessed as unrelated to the study treatment. Mild constipation was the most commonly reported AE, by 3 (10%) active treatment subjects, with 1 subject each in the 375 mg, 525 mg, and 375 mg (SMEDD ingested immediately following dilution) cohorts reporting 1 event each and was considered by the PI to be possibly related to the study.

In Part 2 headache was the most reported AE, reported 11 times by 8 subjects (44%). Fatigue and oropharyngeal pain were reported 3 times by 3 subjects (17%); dyspepsia, myalgia, nausea, and dizziness were reported 2 times by 2 subjects (11%). Events were generally distributed across treatment conditions, with a moderate increase seen in AEs in the combination rencofilstat plus TDF treatment (12/18 subjects [67%]) than in rencofilstat (8/18 [44%]) or TDF (7/18 [39%]) alone.

In Part 3, 2 subjects were discontinued due to AEs (1 due to urinary tract and yeast infections and 1 due to headache and lower abdominal pain). Only 3 AEs occurred in ≥ 3 subjects: headache in 4 subjects (12%); fatigue, and diarrhoea in 3 subjects (9%). All events were Grade 1 or 2 in severity. There was no apparent relationship between rencofilstat dose level and AE frequency.

Overall, there was no trend observed for AE reporting in relation to rencofilstat dose. There were no remarkable observations in the remaining safety assessments for vital signs, 12-lead ECGs, or safety laboratory measurements in this study.

In study CRV431-103, there were no SAEs or deaths reported following treatment with 75 mg rencofilstat alone or in combination with 400 mg ketoconazole or 2 mg midazolam. One patient discontinued study participation due to a TEAE of elevated ALT/AST and subsequent COVID-19 infection. Twenty-eight AEs were recorded for 12 subjects. Investigators assessed 21 AEs as Grade 1, 6 AEs as Grade 2, and 1 AE as Grade 3. The grade 3 AE, elevated ALT, was considered unrelated to study drug and instead related to COVID-19 infection. TEAEs reported in 2 or more subjects were nausea (4 subjects [14.8%], diarrhoea, dizziness, headache, alanine aminotransferase increased, and aspartate aminotransferase increased (2 subjects [7.4%]). There were no findings or changes from baseline observed in any of the laboratory parameters (haematology, coagulation, blood chemistry, urinalysis), vital signs, pulse oximetry or ECG that were deemed to be clinically significant by the investigator.

In study CRV431-104, there was 1 AE related to clinically significant out-of-range vital signs (blood pressure increased) reported by 1 subject in the fed cohort. The AE was Grade 1 (mild) and judged by the Investigator as not related to study treatment.

Overall, rencofilstat was safe and well tolerated at all combinations tested to date in healthy subjects. There are no important identified risks or potential identified risks from the rencofilstat development program.

Non-clinical genotoxicity studies were all negative. These data presented a favourable safety profile for rencofilstat with acceptable safety margins that support the proposed clinical development program. No reproductive or developmental toxicity studies of rencofilstat have been performed as of the IB cut-off date of 01 Oct 2022. However, no females will take part in this study, and the male volunteers must agree to adhere to the contraception requirements defined in Section 9.4. No phototoxicity studies have been conducted on rencofilstat as of the IB cut-off date of 01 Oct 2022. Owing to the unavailability of phototoxicity data, subjects will be advised to minimise exposure to sunlight following dosing until discharge from the study (see Section 9.6).

All formulation excipients are United States Pharmacopeia (USP) grade or National Formulary (NF) grade and therefore have a Generally Recognised As Safe (GRAS) designation. Consequently, it is deemed safe to use in this study.

Given the current safety results, the benefit-risk is anticipated to be favourable at all dose levels tested.

6.4.2 Risks Associated with Administration of Radioactivity

The dosage form will contain a radionuclide, not more than (NMT) 6.4 MBq ^{14}C, so subjects will be exposed to ionising radiation. The effective dose that each subject will receive from 1 administration of 6.4 MBq ^{14}C will not exceed 2.0 mSv. This is approximately 9 months of the average radiation exposure received in the UK each year (2.7 mSv; data obtained from UK Health Security Agency Ionising Radiation Exposure of the UK Population: 2010 Review [13]) and is slightly more (1.1 times) than the radiation dose that would result from 4 abdominal X-rays (0.47 mSv each). It is believed that any increase in the amount of radiation that is received above natural radiation carries a risk of later developing serious and possibly fatal conditions. The risk associated with the maximum possible dose of radiation in this study is very small indeed and is considered to be acceptable.

Extrapolation of data from epidemiological studies of cancers induced by radiation exposure indicates that the risk factor for an adult UK population of both sexes (age range 18 to 64) is 5×10^{-2} per Sv [14]. From this it can be estimated that the lifetime risk of inducing a fatal cancer in a healthy individual from the total exposure of 2.0 mSv is approximately 1 in 10,000. The lifetime risk for being diagnosed with cancer in the UK is around 1 in 2 [15], indicating that this additional risk from this amount of radiation exposure is minimal.

6.4.3 COVID-19 Related Risks and Risk Mitigation Measures

The following risks and risk mitigating measures apply to the time in which the study is conducted during the COVID-19 pandemic.

6.4.3.1 IMP Related Risk

Against the background of the COVID-19 pandemic, the potential risk of a subject developing COVID-19 has been considered in terms of the risk-benefit evaluation. The mode of action of the IMP – as a cyclophilin inhibitor – has been considered alongside available pre-clinical and clinical data (including class effects) and it is considered that a subject would not be at increased risk of either becoming infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; the virus that causes COVID-19) or experiencing a more severe illness. That is, the IMP has no known immunomodulatory effect that would confer an increased risk to healthy subjects enrolled in the study.

6.4.3.2 General COVID-19 Related Risk Mitigation Measures

General risk mitigation against COVID-19 will be implemented in accordance with Quotient's monitoring and prevention control measures.

COVID-19 testing may be performed based on current infection rates and availability of tests. If required, it is planned that testing will comprise an antigen test performed on Day -1 prior to admission to the clinical unit and at return visits. Testing time points may be changed, and additional time points may be added throughout the study as required. The decision on COVID-19 testing and the definition of the testing time points are subject to change based on the current risk mitigation in place and will be agreed by the study team and documented in the Investigator Site File (ISF) via the Clinical Kick-Off Meeting minutes.

The risk mitigation measures, where applicable, will be amended based on emerging government guidance.

6.4.3.3 COVID-19 Vaccine-Related Risk

Approved (including health authority conditional marketing authorisation) COVID-19 vaccines eg killed, inactivated, peptide, DNA and RNA vaccines may be permitted according to the investigator's discretion and as per local guidance.

Based on the mechanism of action of the IMP, as an inhibitor of cyclophilin, there is no perceived impact on the safety of the study subjects or on the study objectives for subjects who may receive these vaccines. It is also very unlikely that administration of the IMP would interfere with COVID19 vaccination response; however, no specific preclinical or clinical investigations have been conducted at this point with rencofilstat.

6.4.4 General Risks and Overall Risk-Benefit Assessment

Collecting a blood sample from a vein may cause pain, swelling, bruising, light-headedness, fainting, and very rarely, clot formation, nerve damage and/or infection at the site of the needle stick.

During cannulation, more than one attempt may be needed to insert the cannula in a vein of a subject and it is possible that bruising and/or inflammation may be experienced at the site of cannulation.

ECG stickers on the subjects' chests and limbs may cause some local irritation and may be uncomfortable to remove but subjects will be closely monitored to ensure any local irritation does not persist.

There is no benefit to the subjects from taking part in this study. The development of a product to treat NASH and viral hepatitis-induced liver disease may be of benefit to the patients with these diseases.

The overall risk benefit balance is therefore considered to be acceptable.

7 Objectives and Endpoints

Objectives	Endpoints
Primary To determine the mass balance recovery after a single oral dose of [^{14}C]-rencofilstat	Mass balance recovery of total radioactivity (TR) in all excreta (urine and faeces): CumAe and Cum%Ae
To determine % parent drug unchanged versus metabolites	Parent to total radioactivity ratio for whole blood based on AUC
To perform metabolite profiling and structural identification from whole blood, urine and faecal samples[a]	Collection of whole blood, urine and faeces samples for metabolite profiling, structural identification, and quantification analysis
Secondary To determine the routes and rates of elimination of [^{14}C]-rencofilstat	Mass balance recovery of TR in urine and faeces separately: Ae, %Ae, CumAe and Cum%Ae by interval
To identify the chemical structure of each metabolite accounting for more than 10% of circulating TR or accounting for 10% or more of the dose in excreta	Identification of the chemical structure of each metabolite accounting for more than 10% by AUC of circulating TR or accounting for 10% or more of the dose in excreta
To evaluate the extent of distribution of TR into blood cells	Evaluation of whole blood:plasma concentration ratios for TR
To provide additional safety and tolerability information for rencofilstat	To provide additional safety and tolerability information for rencofilstat by assessing: incidence of AEs, physical examinations and change from baseline for vital signs, ECGs, and laboratory safety tests
To further explore the oral PK of rencofilstat	PK parameters for rencofilstat in whole blood and TR in plasma and whole blood following a single oral dose, including but not limited to: Tlag, Tmax, Cmax, AUC(0-last), AUC(0-inf), AUC(0-24), Lambda-z, T1/2, CL/F, Vz/F, MRT(0-last) and MRT(0-inf) as applicable

[a] Metabolite profiling and identification will be reported separately from the clinical study report as a standalone document.

8 Study Design

8.1 Study Plan

This is a single centre, open-label, non-randomised, single period, single dose study in healthy male subjects designed to assess the mass balance recovery, PK, metabolite profile and metabolite identification of rencofilstat. It is planned to enrol a single cohort of 6 subjects.

All subjects will receive a single 225 mg oral dose of [^{14}C]-rencofilstat, as a SMEDDS oral emulsion, containing NMT 6.4 MBq, in the fasted state.

Subjects will undergo preliminary screening procedures for the study at the screening visit (Day -28 to Day -2). Subjects will be admitted in the evening on the day before dosing (Day -1).

Whole blood, plasma, urine and faeces samples will be collected at regular intervals for PK analysis, TR analysis, metabolite profiling, mass balance and safety as applicable, from pre-dose to discharge from the clinical unit. Urine and faeces samples may be collected at return visits or home visits if mass balance criteria has not been met by a subject.

Subjects will be dosed on the morning of Day 1 following an overnight fast (minimum 8 h) and will remain resident in the clinical unit until up to 504 h after dosing (up to Day 22).

It is planned that subjects will be released as a group when all subjects have achieved a mass balance cumulative recovery of >90% or if <1% of the dose administered has been collected in urine and faeces within two separate, consecutive 24 h periods. This may result in the subjects being discharged as a group prior to completion of the planned residency period.

If the mass balance criteria are achieved during the planned residency period, collection of all samples (blood, urine and faeces) will be stopped once the current 24 h collection period is complete, and subjects will undergo discharge assessments.

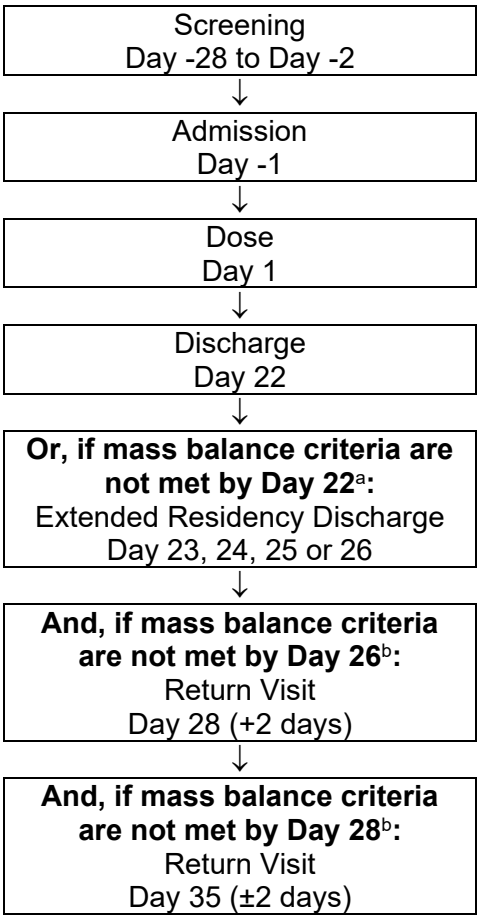
If the mass balance criteria have not been met by all subjects on the morning of Day 22, the residency period for the subjects not achieving the mass balance criteria may be extended up to a maximum of 96 h (Day 26; 600 h post-dose). Only urine and/or faecal samples will be collected during the extended residency period.

If the mass balance criteria have not been met by all subjects on the morning of Day 26, subjects not achieving the release criteria may be required to make up to 2 return visits (each of 24 h duration) at (nominally) Day 28 (+2 days) and Day 35 (±2 days) for additional collection of excreta samples (urine and/or faecal samples).

If the mass balance criteria have not been met following the return visits, then home collections of urine and/or faeces may be requested at the discretion of the investigator for individual subjects.

A subject will be considered evaluable if they have provided mass balance and PK samples for up to 21 days after drug administration or have demonstrated >90% mass balance recovery or have <1% of the administered dose eliminated in excreta for two consecutive days, whichever is sooner.

Figure 1 Study Sequence



[a] If all subjects have not achieved a mass balance cumulative recovery of >90% or if a total of <1% of the dose administered has not been collected in urine and faeces within 2 separate, consecutive 24 h periods on the morning of Day 22, the residency period for the subjects not achieving the mass balance criteria may be extended up to a maximum of 96 h (Day 26). Only urine and/or faecal samples will be collected during the extended residency period.

[b] If the mass balance criteria have not been met by all subjects on the morning of Day 26, subjects not achieving the release criteria may be required to make up to 2 return visits (each of 24 h duration) at (nominally) Day 28 (+2 days) and Day 35 (±2 days) for additional collection of excreta samples (urine and/or faecal samples). If the mass balance criteria have not met following the return visits, then home collections of urine and/or faeces may be requested at the discretion of the investigator for individual subjects.

8.2 Criteria for In-Study Decisions

Not applicable for this study.

8.3 Subject Withdrawal

If a subject wishes to leave the study at any time, they will be permitted to do so. Every reasonable effort will be made by Quotient to complete a final assessment/discharge procedures. Quotient will advise the sponsor of the withdrawal of any subject from the study.

Early withdrawal is defined as the date of the decision to withdraw the subject from the study. Subject completion is defined as the date of the last procedure conducted or last contact (eg unscheduled phone call or visit) for that subject.

Note that if a subject requests to leave the clinical unit earlier than the planned discharge time, eg due to unforeseen personal circumstances, they may be withdrawn from the study and may not be able to return to the clinical unit to complete the study.

This is a single dose study, therefore, after an individual subject has received a dose of IMP, withdrawal of that subject from further dosing is not possible. Subjects will be monitored for the following criteria which may require their withdrawal from some or all study procedures if continuation is not in their best interests, except when the withdrawal is a result of withdrawal of consent:

- Experiencing a serious or severe AE including but not limited to:
    - corrected QT interval by Fridericia's formula (QTcF) of >500 msec or increase in QTcF interval of >60 msec from baseline (confirmed following a repeat ECG)
    - ALT concentration >3 × the upper limit of the reference range (confirmed following a repeat ALT blood test)
- Termination of the study
- Upon the subject's request (withdrawal of consent)
- Significant deviation from the protocol
- Concurrent illness that would adversely affect subject safety or data integrity or requirement for prohibited medication
- At the discretion of the investigator

For the purpose of withdrawal criteria, baseline will be considered as the last available assessment prior to IMP dose.

For a subject who withdraws because of an IMP-related AE, every effort will be made to ensure the subject completes follow-up procedures.

Early withdrawal of consent by the subject to participate in any further activities will be distinguished from withdrawal for any of the other above reasons.

8.4 Subject Replacement

No replacement subjects are planned for this study.

8.5 Stopping Criteria

The study will be halted, and the risk to other subjects evaluated if any of the following criteria are met:

- A serious adverse reaction (ie a SAE considered at least possibly related to the IMP administration) in one subject.
- Severe non-serious adverse reactions (ie severe non-serious AE considered as, at least possibly related to the IMP administration) in two subjects in the same cohort, independent of within or not within the same system organ class.

Relatedness to IMP will be determined by the investigator.

If the study is halted, a temporary halt will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) and ethics committee (EC) in the form of a substantial amendment. The study may be resumed or terminated; however, it will not be resumed until a further substantial amendment to resume the study is submitted and approved by MHRA and EC.

The ARSAC Practitioner will also be informed of the temporary halt.

8.6 Study Termination

After the start of protocol activities but prior to the commencement of dosing, the study may be terminated by the sponsor and investigator without consultation with the MHRA, EC, ARSAC practitioner or ARSAC. The ARSAC practitioner and ARSAC (if ARSAC research application has been submitted or approved) must be notified promptly, that the study will no longer be taking place. Notification of early termination should also be provided to the EC and MHRA within 15 days, clearly explaining the reasons for the termination.

If the study is abandoned prior to commencement of any protocol activities, the investigator or sponsor must notify the EC, MHRA, ARSAC practitioner and ARSAC (if ARSAC research application has been submitted or approved) outlining the reasons for abandonment of the trial.

New information regarding the safety of the IMP that indicates a change in the risk/benefit profile for the compound, such that the risk/benefit is no longer acceptable for subjects participating in the study may also mean termination of the study prior to dosing.

Once exposure to dosing has begun, the study will be completed as planned unless the following criteria are satisfied that require a temporary halt or early termination of the study.

- The occurrence of serious or severe AE(s), as defined in Section 8.5, if considered to be related to the IMP, as defined in Section 14.2, on the dosing day.
- Significant violation of Good Clinical Practice (GCP) that compromises the ability to achieve the primary study objectives or compromises subject safety.

If any of the above occurs, the study will be terminated if careful review of the overall risk/benefit analysis described in Section 6.4 demonstrates that the assumptions have changed and that the overall balance is no longer acceptable. In these circumstances, termination can only take place with the agreement of the investigator and sponsor. The MHRA, EC, ARSAC practitioner and ARSAC will be informed of study termination.

If it becomes necessary to consider termination of the study on the dosing day, dosing may be suspended pending discussion between the investigator, sponsor and ARSAC practitioner (where discussion is related to administration of radiation). Dosing will be stopped immediately on safety grounds.

The study may be terminated or suspended at the request of the MHRA or EC.

8.7 Lost to Follow-up

A subject will be considered lost to follow-up if they fail to return for scheduled visits and cannot be contacted by the clinical unit.

If a subject fails to return to the clinical unit for a required study visit:

- The clinical unit must attempt to contact the subject and reschedule the missed visit as soon as possible.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (eg three telephone calls on three separate occasions and, if necessary, an email or letter to the participant's last known email/postal address). These contact attempts should be documented in the subject's source.
- If the subject cannot be contacted, they will be considered lost to follow-up.

8.8 Treatment Assignment

This is an open-label, non-randomised study, therefore a randomisation schedule will not be produced. Instructions to dispense and dose will be produced prior to dosing with IMP, which will dictate the order in which the treatments should be administered to each subject. The instructions to dispense and dose will be retained in the ISF.

8.8.1 Subject Numbers

Subject numbers will be allocated on the morning of dosing according to the code 001 to 006 using the lowest number available.

8.8.2 Blinding

This is an open-label, non-randomised study and therefore blinding is not required.

9 Selection of Subjects

Subjects will be recruited from the Quotient panel or by direct advertising to the public.

Before subjects are admitted to the clinical unit, The Over Volunteering Prevention System (TOPS) will be checked to ensure that each subject has not been dosed in a study within 90 days of the planned dosing date of this study.

9.1 Informed Consent

Subjects will be provided with a written explanation of the study at least the day before the screening visit. Additionally, subjects may be provided with an information video before the screening visit that introduces them to the study. In the video and/or during the screening visit, a physician or nurse will explain to each subject the nature of the study, its purpose, expected duration and the benefits and risks involved in study participation. Subjects will be informed that, for safety reasons, brief details of their involvement in the study may be revealed to other units and companies that carry out clinical studies nationally. Subjects will then be given the opportunity to ask questions and will be informed of their right to withdraw from the study without prejudice. After this explanation and before entering the study, the subject will voluntarily sign an informed consent form (ICF). Until written consent has been obtained from the subject no study specific procedure or investigation will be performed. If an amendment is made to the participant information sheet (PIS), participants will be re-consented to the most current version of the ICF(s) where appropriate.

9.2 Inclusion Criteria

1. Healthy males
2. Aged 30 to 65 years inclusive at the time of signing informed consent

3. Body mass index (BMI) of 18.0 to 35.0 kg/m^2 as measured at screening
4. Must be willing and able to communicate and participate in the whole study
5. Must have regular bowel movements (ie average stool production of ≥1 and ≤3 stools per day)
6. Must provide written informed consent
7. Must agree to adhere to the contraception requirements defined in Section 9.4

Inclusion criteria 1, 4, 5 and 7 from the list above will be re-assessed at admission or pre-dose.

9.3 Exclusion Criteria

1. Subjects who have received any IMP in a clinical research study within the 90 days prior to Day 1, or less than 5 elimination half-lives prior to Day 1, whichever is longer
2. Subjects who are, or are immediate family members of, a study site or sponsor employee
3. Evidence or history of current SARS-CoV-2 infection within 4 weeks prior to IMP administration
4. History of any drug or alcohol abuse in the past 2 years prior to screening
5. Regular alcohol consumption in males >21 units per week (1 unit = ½ pint beer, or a 25 mL shot of 40% spirit, 1.5 to 2 units = 125 mL glass of wine, depending on type)
6. A confirmed positive alcohol breath test at screening or admission
7. Current smokers and those who have smoked within the last 12 months prior to screening
8. A confirmed breath carbon monoxide reading of greater than 10 ppm at screening or admission
9. Current users of e-cigarettes and nicotine replacement products and those who have used these products within the last 12 months prior to screening
10. Radiation exposure, including that from the present study, excluding background radiation but including diagnostic x-rays and other medical exposures, exceeding 5 mSv in the last 12 months or 10 mSv in the last 5 years. No occupationally exposed worker, as defined in the Ionising Radiation Regulations 2017 [16], shall participate in the study
11. Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the investigator or delegate at screening
12. Clinically significant abnormal clinical chemistry, haematology or urinalysis as judged by the investigator (laboratory parameters are listed in Appendix 1). Subjects known to have Gilbert's syndrome are excluded
13. Confirmed positive drugs of abuse test result (drugs of abuse tests are listed in Appendix 1)
14. Positive hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCV Ab) or human immunodeficiency virus (HIV) antibody results
15. Evidence of renal impairment at screening, as indicated by an estimated eGFR of <60 mL/min/1.73m^2 using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI; 2009) equation
16. History of clinically significant cardiovascular, renal, hepatic, dermatological, chronic respiratory or gastrointestinal disease, malabsorptive, neurological or psychiatric disorder, as judged by the investigator
17. Serious adverse reaction or serious hypersensitivity to any drug or the formulation excipients

18. Presence or history of clinically significant allergy requiring treatment, as judged by the investigator. Hay fever is allowed unless it is active
19. Donation of blood or plasma within the previous 3 months or loss of greater than 400 mL of blood
20. Subjects who have taken known strong or moderate CYP3A4 inducers in the 30 days before IMP administration
21. Subjects who are taking, or have taken, any prescribed or over-the-counter drug or herbal remedies (other than up to 4 g of paracetamol per day) in the 14 days before IMP administration (see Section 11.4). Exceptions may apply, as determined by the investigator, if each of the following criteria are met: medication with a short half-life if the washout is such that no pharmacodynamic activity is expected by the time of dosing with IMP; and if the use of medication does not jeopardize the safety of the trial subject; and if the use of medication is not considered to interfere with the objectives of the study
22. Subjects who have had a COVID-19 vaccine 7 days before dosing
23. Failure to satisfy the investigator of fitness to participate for any other reason

Exclusion criteria 3, 6, 8, 9, 13, 18, 20, 21 and 22 from the list above will be re-assessed at admission/pre-dose.

Healthy subjects who do not meet the inclusion/exclusion criteria for the study will not be enrolled.

9.4 Contraception and Restrictions

Male Subjects with Partners of Childbearing Potential

Male subjects who are sexually active with a partner of childbearing potential must use, with their partner, a condom plus an approved method of highly effective contraception from the time of informed consent until 111 days after IMP administration. This has been calculated based on 90 days (one cycle of spermatogenesis) plus 5 half-lives of the IMP. 5 half-lives have been calculated as 21 days based on the mean half-life of 98.138 h observed in healthy volunteers at a single dose of 225 mg.

The following methods are acceptable:

- Partner's use of combined (oestrogen and progestogen-containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- Partner's use of progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable/implantable
  - intrauterine hormone-releasing system
- Partner's use of intrauterine device
- Vasectomised
- Partner's bilateral tubal occlusion

These contraception requirements are considered to be more conservative than the guidance issued by the Heads of Medicines Agency: Clinical Trials Facilitation Group [17].

Males with Partners of Non-childbearing Potential

There is a significant risk of drug exposure through the ejaculate (which also applies to vasectomised males) that might be harmful to sexual partners. Therefore, even if a male is sexually active with a partner of non-childbearing potential they will be required to use a condom from IMP administration until discharge from the study.

All Subjects

Alternatively, sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

9.4.1 Sperm Donation

Male subjects should not donate sperm for the duration of the study and for 111 days after IMP administration.

9.5 Pregnancy

Subjects will be instructed that if their partner becomes pregnant during the study this should be reported to the investigator. The investigator should also be notified of pregnancy occurring during the study but confirmed after completion of the study. In the event that a subject's partner is subsequently found to be pregnant after the subject is included in the study, then consent will be sought from the partner and, if granted, any pregnancy will be followed and the status of mother and/or child(ren) will be reported to the sponsor after delivery.

A pregnancy notification form and follow-up will be completed.

9.6 Additional Study Restrictions

The following additional restrictions will be in place for the duration of the study:

1. Subjects must abstain from alcohol during the 24 h prior to screening and the 24 h prior to admission until discharge from the clinical unit
2. Subjects must not drink liquids or eat food containing grapefruit, cranberry, caffeine or other xanthines from 24 h prior to admission until discharge from the clinical unit
3. Subjects should refrain from eating food containing poppy seeds for 48 h prior to screening and for 48 h prior to admission until discharge from the clinical unit
4. Subjects must not take part in any unaccustomed strenuous exercise from the 72 h before the screening visit and then from 72 h prior to admission until discharge from the study
5. Must not donate blood or plasma (outside of this study), from screening, throughout the study duration, and for at least 90 days following dose of IMP
6. Due to the unavailability of phototoxic data for rencofilstat, subjects will be advised to minimise exposure to sunlight by spending a reduced amount of time outdoors/remaining inside the clinical unit during the residential periods of the study, wearing sunglasses and clothing that minimises exposure to sunlight and using sun cream on exposed areas when going outdoors. They will also be advised not to use sunbeds. This restriction will be advised from dosing until 7 days post-dose

7. Subjects will not be permitted to shower for 24 h post-dose (to ensure the collection of all samples)

The additional restrictions above are not exclusion criteria; if non-compliance occurs, a protocol deviation will be completed.

10 Study Procedures

Study procedures will be performed as detailed in the study schedule of assessments in Appendix 2, and in accordance with Quotient's standard operating procedures (SOP)s unless otherwise stated in this protocol.

10.1 Screening

Within the 28 days preceding dosing, all subjects will be required to undergo a screening visit. Screening procedures will be carried out in accordance with the schedule of assessments in Appendix 2.

If the start of the study is delayed for any reason so that the interval between screening and dosing exceeds 28 days, all or part of the screening procedures will be repeated at the discretion of the investigator.

Screening safety procedures such as safety bloods, ECGs, vital signs, carbon monoxide breath tests, alcohol breath tests and urinalysis can be repeated as clinically indicated under the discretion of the investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the trial.

10.1.1 Subject Re-Screening

This study permits the re-screening of a subject who has discontinued the study as a pre-treatment failure (ie subject has not been treated); the reason for failure must be temporary and expected to resolve. If re-screened, the subject must be re-consented.

10.2 Admission and Pre-dose Procedures

The identity of the subjects will be confirmed at admission and pre-dose.

In addition, the ongoing eligibility of subjects will be re-assessed at admission/pre-dose, as described in Sections 9.2 and 9.3.

Admission/pre-dose safety procedures such as safety bloods, ECGs, vital signs, urinalysis and drugs of abuse tests can be repeated as clinically indicated under the discretion of investigator or sub-investigator if there is a concern regarding a subject's safety or eligibility to participate in the clinical trial.

If dosing is delayed, subjects who have completed admission procedures do not need admission procedures to be repeated if dosing is within 2 days and the subjects have remained resident in the clinical unit.

The subjects will be admitted to the clinical unit on the evening before dosing (Day -1).

In addition, subjects may be required to visit the clinical unit on Day -1 prior to admission to the clinical unit for SARS-CoV-2 antigen tests (if required; see Sections 6.4.3.2 and 14.5.7). Test results must be available prior to dosing.

The admission and pre-dose procedures are presented in Appendix 2.

10.3 Study Day Procedures

10.3.1 Blood Volume

The total blood volume for each subject will not exceed 550 mL in a 4-week period.

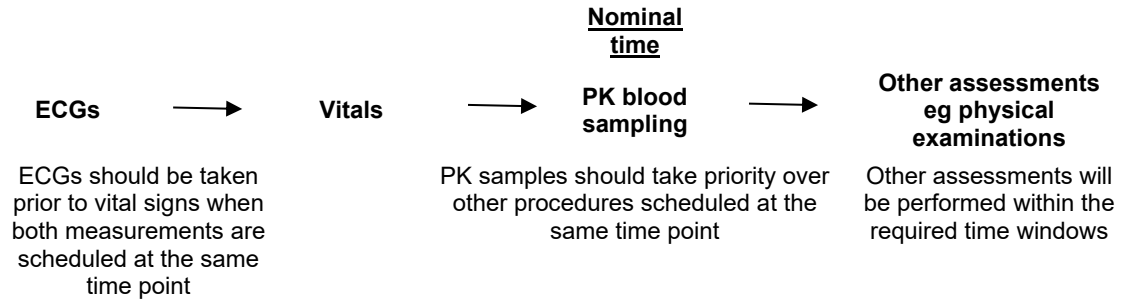
The first 0.5 mL of blood withdrawn via cannula will be discarded.

10.3.2 Timing of Procedures

There are times where the protocol requires more than one procedure to be completed at the same time point. In these instances, the following will apply to post-dose time points:

- PK samples should take priority over other procedures scheduled at the same time point
- ECGs should be taken prior to vital signs when both measurements are scheduled at the same time point
- Other assessments, eg physical examinations, will be performed within the required time windows

As guidance, the preferred order of assessments is:



All safety assessments will be timed and performed relative to the start of dosing.

10.3.3 Discharge from the Clinical Unit

A subject will be allowed to leave the premises without additional investigator or sub-investigator review, following completion of study-specific procedures providing that:

- No AEs have been reported during the study visit
- The subject responds in the affirmative when asked if they are feeling well

Refer to Section 8.1 for further details of subject discharge.

If any of these conditions are not met, then the subject will only be allowed to leave the clinical unit with the authorisation of the investigator or sub-investigator.

There will be no continued provision of the study intervention or treatment for subjects as this study involves healthy volunteers only.

10.3.4 Return Visits

If the mass balance criteria have not been met by the morning of Day 26, subjects not achieving the release criteria may be required to return to the clinical unit for up to 2 return visits on Day 28 (+2 days; 648 h post-dose) and (if mass balance criteria are still not met by Day 28) Day 35 (±2 days; 816 h post-dose) for 24-h faeces and/or urine collections as described in Appendix 2.

Refer to Section 8.1 for further details.

10.3.5 Medical Supervision

A physician will be responsible for the clinical aspects of the study and will be available at all times during the study. In accordance with the current Association of the British Pharmaceutical Industry guidelines [18], each subject will receive a card stating the telephone number of the investigator and the 24/7 contact details of the Quotient's on-call physician.

11 Dosing of Subjects

11.1 Food and Fluid Intake

Subjects will be allowed water up to 1 h before the scheduled dosing time and will be provided with 240 mL of water at 1 h post-dose (to ensure adequate hydration), and thereafter water will be allowed ad libitum after 1 h post-dose. Decaffeinated fluids will be allowed ad libitum from lunch time on the day of dosing.

If, for technical reasons, dosing is delayed for more than 2 h beyond the expected dosing time, subjects will receive 200 mL of an electrolyte drink (eg Lucozade Sport) at the originally scheduled dosing time, or earlier if possible.

The calorie/fat content of meals are not required to be controlled. Subjects will be provided with a standardised menu.

Subjects will be provided with a light snack and then fast from all food and drink (except water) for a minimum of 8 h on the day prior to dosing until approximately 4 h post-dose at which time lunch will be provided. An evening meal will be provided at approximately 10 h post-dose and an evening snack at approximately 14 h post-dose. On subsequent days, meals will be provided at appropriate times.

If an individual subject has not experienced a bowel movement in any 36 h period post-dose, fluid intake should be increased and administration of a mild laxative (eg prune juice or a mild stool softener) should be implemented.

11.2 Administration of Test Preparations

Specific details of the IMP and doses to be administered are provided in Section 5.2 and Section 8.1, respectively. Subjects will be dosed on the morning of Day 1.

The exact time of dosing will be decided based on logistics and will be documented in the source.

Subjects will receive 1 single administration on a single occasion.

Immediately after administration of the oral emulsion, the dosing vessel will be rinsed with water and subjects will consume the rinse solution. Subjects will then consume further water to a total volume of 240 mL (including the dosing volume and volume used to rinse the dosing vessel).

11.3 Dosing Compliance

During all clinical phases of the study, subjects will be observed by study staff to assure compliance to all study procedures, including dose administration.

Mouth checks will be conducted after dosing to ensure the emulsion has been swallowed.

The date and time that each subject is dosed will be recorded in the subject's source data. Any violation of compliance will require evaluation by the investigator and sponsor to determine if the subject can continue in the study.

11.4 Prior and Concomitant Medications

No prescribed, over-the-counter medication or herbal remedies will be permitted from 14 days before IMP administration until discharge from the study except up to 4 g of paracetamol per day and those deemed necessary by the investigator to treat AEs (see also Section 9.3).

In addition, no strong or moderate CYP3A4 inducers will be permitted from 30 days before IMP administration until discharge from the study.

Any medications used will be recorded in the source.

Subjects are not permitted to receive the COVID-19 vaccine within 7 days prior to administration of IMP, so that by the time of dosing any effects of the vaccine (eg, pyrexia, fatigue, pain/stiffness at site of injection) are likely to have abated. Subjects will be permitted to have the vaccination when discharged from the clinical unit.

Emergency equipment and drugs will be available within the clinical unit as per current standard procedures. In the unlikely event that they are required, their use will be documented.

12 Assessment of Efficacy

Not applicable for this Phase I study.

13 Assessment of Mass Balance, Pharmacokinetics, Metabolite Profiling and Identification, and Pharmacodynamics

13.1 Assessment of Mass Balance, Pharmacokinetic, and Metabolite Profiling and Identification

Mass balance, PK, and metabolite profiling and identification assessments will be performed as detailed in the study schedule of assessments in Appendix 2.

13.1.1 Pharmacokinetic and Metabolite Profiling and Identification Blood Sampling

Venous blood samples will be collected from the subjects by a trained member of the clinical team. Consent will be collected from the subjects for use of these samples for the purposes of the proposed study. Samples will be processed to isolate plasma and PK analysis will be carried out on plasma (TR) and whole blood (rencofilstat, TR, metabolite profiling and identification) samples.

Plasma and whole blood samples will be sent for laboratory testing in linked anonymised form (subject number only). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor, however not by the laboratory staff or sponsor.

Venous blood samples will be withdrawn via an indwelling cannula or by venepuncture according to the time schedule presented in Appendix 2.

All PK and metabolite profiling and identification windows will be timed relative to the start of dosing.

The acceptable deviations from the nominal blood sampling times are as follows:

- The pre-dose samples will be taken ≤1 h before dosing
- 0 to 1 h post-dose samples will be taken within ± 2 min of the nominal post-dose sampling time
- 1.5 h to 12 h post-dose samples will be taken within ± 10 min of the nominal post-dose sampling time
- 16 h to 504 h post-dose samples (or up to 600 h post-dose if the residency period is extended) will be taken within ± 30 min of the nominal post-dose sampling time

Samples will be collected into appropriate tubes as specified by the bioanalytical laboratory. Details of sample tubes and processing will be contained in the Clinical Sample Processing Manual (CSPM).

Samples will be shipped to Pharmaron UK for the analysis of rencofilstat, metabolite profiling and TR.

13.1.2 Mass Balance and Metabolite Profiling and Identification Urine Sampling

Urine samples will be collected according to the time schedule presented in Appendix 2.

A single urine sample will be taken at pre-dose (or the first void of the day). Where a sample is not provided, this will not be considered a deviation. All individual urine voids will be collected and shipped to the metabolism laboratory for analysis, according to Quotient's SOPs, unless indicated otherwise by the sponsor.

Consent will be collected from the subjects for use of these samples for the purposes of the proposed study. Samples will be collected into appropriate containers as specified by the bioanalytical laboratory. Details of sample containers and processing will be contained in the CSPM.

Urine samples will be sent for laboratory testing in linked anonymised form (subject number). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor; however, not by the laboratory staff or sponsor.

Samples will be shipped to Pharmaron for the analysis of metabolite profiling and TR.

13.1.3 Mass Balance and Metabolite Profiling and Identification Faecal Sampling

Faecal samples will be collected according to the time schedule presented in Appendix 2.

The pre-dose faecal sample will be taken between admission and pre-dose. If a pre-dose faecal sample cannot be obtained, the subject will still be dosed. Where a sample is not provided, this will not be considered a deviation.

Consent will be collected from the subjects for use of these samples for the purposes of the proposed study. Samples and toilet paper will be collected into appropriate pots/containers as specified by the metabolism laboratory. Details of sample containers and processing will be contained in the CSPM.

Faecal samples will be sent for laboratory testing in linked anonymised form (subject number). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor; however, not by the laboratory staff or sponsor.

Samples will be shipped to Pharmaron for the analysis of TR and for metabolite profiling.

13.1.4 Unexpected Sources of Elimination

During the study, other accidental sources of elimination will be collected as voided (eg emesis).

Samples will be shipped to the Pharmaron for the analysis of TR.

13.2 Assessment of Pharmacodynamics

Not applicable for this Phase I study.

14 Assessment of Safety

14.1 Definition and Classification of Adverse Events

An AE is any untoward medical occurrence in a subject that occurs either before dosing (referred to as a pre-dose AE) or once a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

An adverse drug reaction is any AE where a causal relationship with the IMP is at least a reasonable possibility (possibly related or related).

AEs will be monitored from the time the subject signs the ICF until discharge from the study. The severity of AEs should be assessed as follows:

Mild An AE that is easily tolerated by the subject, causes minimal discomfort and does not interfere with everyday activities

Moderate An AE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed

Severe An AE that prevents normal everyday activities; treatment or other intervention usually needed

14.2 Assessment of Causality

Every effort should be made by the investigator to try to explain each AE and assess its relationship, if any, to the IMP. The temporal relationship of the event to IMP administration should be considered in the causality assessment (ie if the event starts soon after IMP administration and resolves when the IMP is stopped).

Causality should be assessed using the following categories:

Unrelated: Clinical event with an incompatible time relationship to IMP administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the IMP

Possibly related: Clinical event with a reasonable time relationship to IMP administration, and that is unlikely to be attributed to concurrent disease or other drugs or chemicals

Related: Clinical event with plausible time relationship to IMP administration and that cannot be explained by concurrent disease or other drugs or chemicals

The degree of certainty with which an AE is attributed to IMP administration (or alternative causes, eg natural history of the underlying disease, concomitant therapy) will be determined by how well the experience can be understood in terms of one or more of the following:

- Known pharmacology of the IMP
- Reactions of a similar nature have been previously observed with the IMP or this class of drug
- The experience being related by time to IMP administration
- Alternative cause

14.3 Recording Adverse Events

AEs (including SAEs) will be recorded from the time of providing written informed consent until discharge from the study. During each study visit the subject will be questioned directly regarding the occurrence of any adverse medical event according to the schedule in the source. All AEs, whether ascribed to study procedures or not, will be documented immediately in the subject's source. This will include the date and time of onset, a description of the AE, severity, seriousness, duration, actions taken, outcome and an investigator's current opinion on the relationship between the study drug and the event. A diagnosis and final opinion on the relationship between the study drug and the event will be provided at the end of the study by the investigator.

Any subject who withdraws from the study due to an AE will be followed up until the outcome is determined and written reports are provided by the investigator.

14.4 Serious Adverse Events

14.4.1 Definition of Serious Adverse Events

A SAE is defined as any untoward medical occurrence that at any dose:

- Results in death

- Is life-threatening
- Requires hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect
- An important medical event as recognised by the investigator

14.4.2 Definition of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are AEs that are believed to be related to an IMP and are both unexpected (ie the nature or severity is not expected from the information provided in the IB) and serious. SUSARs are subject to expedited reporting to the MHRA and EC and ARSAC practitioner (when related to radiation exposure) (see Section 16.3.2 for details on reporting SUSARs).

14.5 Laboratory Measurements

Venous blood and urine samples will be collected from the subjects by a trained member of the clinical team. Consent will be collected from the subjects for use of these samples for the purposes of the proposed study.

Blood and urine samples are sent for laboratory testing in linked anonymised form (subject number, gender and date of birth for analytical reasons). This information is able to be linked directly to the volunteer by the Quotient research team and study monitor; however, not by the laboratory staff or sponsor.

Safety laboratory tests and virology will be carried out on blood samples, and drugs of abuse tests and urinalysis will be carried out on urine samples. The research will not involve analysis or use of human DNA.

Blood and urine samples results will be reviewed by a physician and acted upon before the subject is dosed or receives their next dose, or is released from the study, as is appropriate. A list of the laboratory parameters measured is presented in Appendix 1.

14.5.1 Haematology and Clinical Chemistry

Laboratory tests will be performed by The Doctors Laboratory according to the time schedule presented in Appendix 2. Blood samples will be collected and processed as detailed in the CSPM. Scheduled blood samples will be taken following a fast of at least 8 h.

The acceptable deviations from the nominal blood sampling time points for laboratory assessments are:

- The pre-dose blood sample will be taken ≤2 h before dosing
- Post-dose blood samples will be taken ± 1 h from the nominal blood sampling time

eGFR will be calculated at screening by The Doctors Laboratory using CKD-EPI equation [19] for eligibility purposes.

14.5.2 Virology

HBsAg, HCV Ab and HIV 1 and 2 tests will be performed from the clinical chemistry sample (see Section 14.5.1 for sample collection and processing information).

14.5.3 Urinalysis

Urinalysis will be performed on-site using a dipstick according to the time schedule presented in Appendix 2. Urine samples will be collected and processed as detailed in the CSPM. If microscopy is required, a urine sample (approximately 20 mL) will be taken from the urine collection for TR and metabolite profiling and identification and sent to The Doctors Laboratory, after the void has been weighed.

The acceptable deviations from the nominal urine sampling time points for urinalysis are:

- The pre-dose urine sample will be the first void of the day or a sample collected ≤3 h before dosing
- Post-dose urine samples will be taken ± 2 h from the nominal urine sampling time

14.5.4 Drug Screen

A urine drug screen will be performed on-site using a point of care testing method (eg Alere Drug Screen Test Cup) according to the time schedule presented in Appendix 2. The sample will be collected and processed as detailed in the CSPM. Subjects will be screened for the drugs of abuse listed in Appendix 1.

14.5.5 Alcohol Breath Test

An alcohol breath test will be performed according to the time schedule presented in Appendix 2. A positive result will exclude the subject from dosing during that admission.

14.5.6 Carbon Monoxide Breath Test

A carbon monoxide breath test will be performed according to the time schedule presented in Appendix 2. A result of greater than 10 ppm will exclude the subject from the study.

14.5.7 SARS-CoV-2 Tests (If Required)

Testing for the SARS-CoV-2 virus may be performed based on current infection rates and availability of tests. Tests will be performed according to the time schedule presented in Appendix 2. The samples will be collected and processed as detailed in the CSPM.

Testing time points may be changed and additional time points may be added throughout the study as required. The decision on COVID-19 testing and the definition of the testing time points are subject to change based on the current risk mitigation in place and will be agreed by the study team and documented in the ISF via the Clinical Kick-Off Meeting minutes.

14.5.8 Abnormal Laboratory Findings

In cases where laboratory findings are outside the normal range and the investigator believes that the results may be of clinical significance, repeat sampling may be requested as clinically indicated. If the abnormal finding is clinically significant, appropriate actions will be taken eg the subject will not be entered into the study or the subject may be withdrawn from the study. The subject will be referred to their general practitioner or other appropriate provider for further care if necessary. The same will apply if the results of the HBsAg, HCV Ab or HIV test are positive and in addition the investigator will ensure that adequate counselling is available if requested.

Abnormal results at follow-up assessments will also require repeat testing if the investigator believes the results may be of clinical significance.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

Additional blood and/or urine samples may be taken for safety tests. Furthermore, additional assays outside those specified in the protocol may be performed for safety reasons as requested by the investigator or sub-investigator.

14.6 Vital Signs Measurements

Blood pressure and heart rate will be measured by an automated recorder after the subject has been in a supine position for a minimum of 5 min and oral temperature will be measured according to the time schedule presented in Appendix 2. The acceptable deviations from the nominal vital signs measurement time points are:

- The pre-dose vital signs measurements will be taken ≤2 h before dosing
- Post-dose vital signs measurements will be taken ± 15 min from the nominal post-dose time points
- Discharge vital signs measurements will be taken ± 1 h from the nominal time point
- For return visits vital signs measurements will be taken ± 2 h from the nominal return visit time point

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator or sub-investigator.

Any clinically significant abnormality, including changes from baseline, must be reported as an AE.

14.7 ECG Measurements

Single 12-lead ECGs will be measured after the subject has been in the supine position for a minimum of 5 min according to the time schedule presented in Appendix 2. The acceptable deviations from the nominal ECG measurement time points are:

- The pre-dose ECG measurements will be taken ≤2 h before dosing
- Post-dose ECG measurements will be taken ± 15 min from the nominal post-dose time point
- Discharge ECG measurements will be taken ± 1 h from the nominal time point

If a subject shows an abnormal assessment at any stage, repeat measurements may be made and the abnormality followed to resolution if required. Additional measurements may be taken as deemed necessary by the investigator or sub-investigator.

Any clinically significant abnormality, including changes from baseline, will be reported as an AE.

14.8 Body Weight, Height and BMI

The subject's body weight and height will be measured and their BMI will be calculated according to the time schedule presented in Appendix 2.

14.9 Physical Examination

Subjects will undergo a physical examination according to the time schedule presented in Appendix 2.

In the targeted (symptom driven) physical examination, a physician will assess the subject; if the subject reports feeling unwell or has ongoing AEs, then the physician will examine the appropriate body system(s) if required.

14.10 Additional Safety Procedures

Additional non-invasive procedures that are already specified in the protocol may be performed, if it is believed that an important effect of the IMP(s) is occurring or may occur at a time when no measurements are scheduled, or if extra procedures are needed in the interests of safety.

Additional blood samples for safety assessments may be taken if required by the investigator or sub-investigator at any point.

15 Statistics and Data Analysis

15.1 Sample Size Justification

The study is exploratory and no formal sample size calculation has been made. Based on experience from previous studies of a similar design, a total of 6 subjects are to be enrolled and a minimum of 4 evaluable subjects are considered sufficient.

15.2 Data Management

Data management will be performed by Quotient.

Study data will be managed using a validated electronic case report form (eCRF) database system and subjected to data consistency and validation checks. Data queries will be raised within the study eCRF database by data management staff and resolved with the assistance of clinical staff.

AEs, medical histories and medications will be coded using the Medical Dictionary for Regulatory Activities (version to be specified in the Data Management Plan [DMP]) and the World Health Organization (WHO) Drug Dictionary Global Drug Reference (version to be specified in the DMP), respectively. An independent coding review will also be performed within the Data Sciences department.

Clinical chemistry and haematology data (and other safety laboratory data) will be collected by a central laboratory (The Doctors Laboratory) and stored electronically in their clinical pathology system. The data will be transferred electronically to Quotient and all demographic details and sample dates will be cross-referenced with the corresponding data on the study database. All queries will be resolved with the assistance of laboratory staff, or if necessary, clinical staff.

The database will be closed after all queries have been resolved. The database will be locked when all criteria listed in the DMP are met.

Further details are addressed in the DMP.

15.3 Mass Balance, Pharmacokinetic, and Metabolite Profiling and Identification Data Analysis

15.3.1 Mass Balance Data Analysis

The mass balance parameters presented in Table 3 will be calculated by Quotient using the concentration data for TR in urine and faeces (and emesis, if applicable) provided by Pharmaron.

Table 3 Mass Balance Parameters

Parameter	Definition
Ae(urine)	amount of TR excreted in urine
%Ae(urine)	amount of TR excreted in urine expressed as a percentage of the radioactive dose administered
CumAe(urine)	cumulative amount of TR excreted in urine
Cum%Ae(urine)	cumulative amount of TR excreted in urine expressed as a percentage of the radioactive dose administered
Ae(faeces)	amount of TR eliminated in faeces
%Ae(faeces)	amount of TR eliminated in faeces expressed as a percentage of the radioactive dose administered
CumAe(faeces)	cumulative amount of TR eliminated in faeces
Cum%Ae(faeces)	cumulative amount of TR eliminated in faeces expressed as a percentage of the radioactive dose administered
Ae(total)	amount of TR excreted in urine and faeces combined
%Ae(total)	amount of TR excreted in urine and faeces combined expressed as a percentage of the radioactive dose administered
CumAe(total)	cumulative amount of TR excreted in urine and faeces combined
Cum%Ae(total)	cumulative amount of TR excreted in urine and faeces combined expressed as a percentage of the radioactive dose administered

Further analysis details will be included in the Reporting and Analysis Plan (RAP).

15.3.2 Pharmacokinetic and Total Radioactivity Data Analysis

The whole blood concentration data for rencofilstat and plasma and whole blood TR concentration data provided by Pharmaron will be analysed by Quotient, using Phoenix WinNonlin v8.3 or a more recent version (Certara USA, Inc., USA).

Calculated rencofilstat concentrations may be corrected for specific activity of the administered radiolabelled oral dose by the Data Sciences department at Quotient.

PK analysis of the whole blood concentration-time data obtained will be performed using appropriate non-compartmental techniques to obtain estimates of the PK parameters presented in Table 4 and where possible and appropriate.

Table 4 Whole Blood Pharmacokinetic Parameters

Parameter	Definition
Tlag	Time prior to the first measurable concentration
Tmax	Time of maximum observed concentration
Cmax	Maximum observed concentration
AUC(0-24)	Area under the curve from time 0 to 24 h post-dose
AUC(0-last)	Area under the curve from time 0 to the time of last measurable concentration
AUC(0-inf)	Area under the curve from time 0 extrapolated to infinity

Parameter	Definition
AUCextrap	Area under the curve from time of the last measurable concentration to infinity as a percentage of the area under the curve extrapolated to infinity
AUC ratio	Parent to total radioactivity ratio for whole blood based on AUC
T1/2	Terminal elimination half-life
Lambda-z	First order rate constant associated with the terminal (log-linear) portion of the curve
CL/F	Total body clearance calculated after a single extravascular administration where F (fraction of dose bioavailable) is unknown
Vz/F	Apparent volume of distribution based on the terminal phase calculated using AUC(0-inf) after a single extravascular administration where F (fraction of dose bioavailable) is unknown
MRT(0-last)	Mean residence time from time 0 to time of the last measurable concentration
MRT(0-inf)	Mean residence time from time 0 extrapolated to infinity

In addition, whole blood-to-plasma TR concentration ratios will be calculated.

Further details of the PK data analysis will be included in the RAP.

15.3.3 Metabolite Profiling and Identification

Metabolite profiling of whole blood, urine and faeces will be performed using liquid chromatography-radio-detection with subsequent mass spectrometry where appropriate.

Identification of the chemical structure of each metabolite accounting for greater than 10% of circulating radioactivity in whole blood (“AUC pool”) and accounting for greater than 10% of the dose in the urine and faeces (from urine pools and faeces homogenate pools) will be performed.

These aspects will be reported separately from the clinical study report as a standalone document.

15.4 Statistical Data Analysis

Production of summary tables, figures and listings for this study will be performed by Quotient using the statistical package SAS (v9.4 or more recent version).

All safety, mass balance recovery and PK data will be listed.

No formal statistical analysis will be performed for this study. Descriptive statistics (eg mean, median, standard deviation, minimum, maximum and number of subjects with an observation [n]) are considered adequate for a study of this type. Additional statistics will be provided for PK-related data, including coefficient of variation (CV%), geometric mean and geometric CV%.

Populations and analysis sets will be determined for safety, PK and mass balance data after database lock when the relevant data are available, using the criteria defined in the RAP; the RAP will be signed off prior to database lock.

Further details relating to the statistical analysis will be included in the RAP including the following:

- Criteria to be used to define each of the population and analysis sets
- Additional detail covering the analyses and/or description of primary and secondary analyses and safety data

- Handling of missing data, unused or spurious data
- Handling of data from withdrawn subjects

15.5 Interim Analysis

No formal interim analyses are planned for this study.

16 Safety Reporting to Regulatory Authorities and Ethics Committees

16.1 Events Requiring Expedited Reporting

SUSARs (Section 14.4.2) are subject to expedited reporting to the appropriate regulatory authority and EC and the ARSAC practitioner when related to radioactive exposure.

In addition to SUSARs, other safety issues may qualify for expedited reporting where they might materially alter the current benefit-risk assessment of an IMP or that would be sufficient to consider changes in the IMPs' administration or in the overall conduct of the study, for instance:

- An increase in the rate of occurrence or a qualitative change of an expected serious adverse reaction, which is judged to be clinically important
- SAEs that occur after the subject has completed the clinical study where the sponsor considers them to be a SUSAR
- New events related to the conduct of the study or the development of the IMPs and likely to affect the safety of the subjects, such as:
    - An SAE which could be associated with the study procedures and which could modify the conduct of the study
    - A major safety finding from a newly completed animal study (such as carcinogenicity)
    - Any anticipated end or temporary halt of a study for safety reasons and conducted with the same IMPs in another country by the same sponsor

16.2 Urgent Safety Measures

If Quotient or any of its staff or contractors becomes aware of an actual or potential urgent safety issue, then the sponsor must be immediately contacted so that appropriate urgent safety measures can be agreed. An urgent safety issue is defined as:

- An immediate hazard to the health or safety of subjects participating in a clinical study
- A serious risk to human health or potentially a serious risk to human health

An urgent safety issue may include issues with an investigational drug or comparators, study procedures, inter-current illness (including pandemic infections), concomitant medications, concurrent medical conditions or any other issues related to the safe conduct of the study or that pose a risk to study subjects.

In exceptional circumstances of imminent hazard and in order to safeguard the health or safety of individuals, Quotient may take urgent safety measures before informing the sponsor, but the sponsor must be informed immediately after the hazard has resolved.

The sponsor is responsible for informing the appropriate regulatory authorities, and the EC; the task of reporting urgent safety measures will be delegated to Quotient.

16.3 Reporting

16.3.1 Reporting Serious Adverse Events

The investigator must notify the study sponsor and pharmacovigilance provider of any SAE or serious adverse reaction immediately, and in all cases within 24 h of becoming aware of the event or reaction. A copy of the written report of the event should promptly be sent to the study sponsor for information purposes, in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines for GCP [20].

16.3.2 Reporting of Suspected Unexpected Serious Adverse Reactions

It is the responsibility of the sponsor to determine whether a reported SAE fits the classification of a SUSAR and to notify the investigator of their decision as soon as possible.

16.3.3 Expedited Reporting of Events

It is the responsibility of the sponsor to determine whether an event requires expedited reporting and to notify the investigator of their decision as soon as possible.

Where expedited reporting is required, the following procedures should be followed.

Fatal or life-threatening SUSARs

It is the responsibility of the sponsor to report fatal or life-threatening SUSARs to the MHRA as soon as possible, but no later than 7 calendar days after they first became aware of the reaction. Any additional relevant information should be sent within 8 days of the report. The task of reporting fatal or life-threatening SUSARs may be delegated to the pharmacovigilance provider.

The sponsor is required to notify the EC of any fatal or life-threatening SUSAR as soon as possible, but no later than 7 calendar days after they first became aware of the reaction. Any additional relevant information should be sent within 8 days of the report. The task of reporting fatal or life-threatening SUSARs may be delegated to the investigator.

The ARSAC Practitioner will be notified of any fatal or life-threatening SUSAR that is considered related to the exposure to radioactivity.

Other SUSARs

It is the responsibility of the sponsor to report other SUSARs to the MHRA as soon as possible, but no later than 15 calendar days after they first became aware of the reaction. The task of reporting other SUSARs may be delegated to the pharmacovigilance provider.

The sponsor is required to notify the EC of other SUSARs as soon as possible, but no later than 15 calendar days after they first became aware of the reaction. The task of reporting other SUSARs may be delegated to the investigator.

The ARSAC Practitioner will be notified of any SUSAR that is considered related to the exposure to radioactivity.

16.3.4 Reporting of Urgent Safety Measures

Quotient is required to notify the MHRA and the EC of an urgent safety measure immediately by telephone and follow-up in writing within 3 calendar days from the date the measures are taken.

16.3.5 Reporting of COVID-19 Vaccine-Related Adverse Events

AEs considered by the investigator to be related to COVID-19 vaccines will be reported to the MHRA via the Yellow Card system.

16.4 Serious Breaches

It is the responsibility of the sponsor to notify the MHRA of any serious breach, which is likely to affect, to a significant degree, the safety or mental integrity of the subjects of the study or the scientific value of the study.

All serious breaches will be notified to the MHRA within 7 days. The reporting will be performed by the party who suspects the serious breach.

17 Protocol Amendments and Deviations

17.1 Amendments

After the protocol has been submitted to the MHRA and EC, any amendment must be agreed by the investigator after discussion with the sponsor and will be formally documented.

All substantial amendments will be submitted to the MHRA and/or EC for approval and/or an opinion, respectively, as required by current regulations. Any amendments relating to the administration of radioactive substances will be reviewed by the ARSAC practitioner prior to submission to ARSAC as required by the current ARSAC Notes for Guidance [12]. The ARSAC practitioner will also be notified of any substantial amendments to the PIS and ICF and/or protocol.

If the PIS and ICF are updated as a result of the substantial amendment, the new approved versions will be used to re-consent currently enrolled subjects and must be provided to newly enrolled subjects prior to their entry into the study.

17.2 Protocol Deviations

The study must be conducted in accordance with the Clinical Protocol. Should a protocol deviation occur, it must be promptly assessed in order to decide whether any of these non-compliances should be reported to the MHRA as a serious breach of GCP and the Clinical Protocol.

Protocol waivers are not acceptable.

Deviations from the protocol will be recorded in the source as noted by the clinical staff. If necessary, the sponsor will be informed of the deviation.

Any protocol deviations assessed as major will be discussed with the sponsor in order to determine if the withdrawal criteria stated in Section 8.3 have been met.

18 Regulatory

18.1 Compliance

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

- ICH GCP Guidelines approved by the Committee for Medicinal Products for Human Use (CHMP) on 17 Jul 1996, which came into force on 17 Jan 1997, updated Jul 2002, Integrated Addendum E6 (R2) dated 09 Nov 2016 [20]
- The Medicines for Human Use (Clinical Trials) Regulations. Statutory Instruments 2004 No. 1031 [21]
- The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2006 No. 1928 [22]
- The Medicines for Human Use (Clinical Trials) Amendment (No. 2) Regulations. Statutory Instruments 2006 No. 2984 [23]
- The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2008 No. 941 [24]
- The Medicines for Human Use (Clinical Trials) (Amendment) (EU Exit) Regulations. Statutory Instruments 2019 No. 744 [25]
- Health and Safety. The Ionising Radiations Regulations 2017. Statutory Instrument 2017 No. 1075 [14]
- Health and Safety. Ionising Radiation (Medical Exposure) Regulations 2017. Statutory Instrument 2017 No. 1322 [16]

In addition, the study will be performed according to the ethical principles outlined in the World Medical Association Declaration of Helsinki and its amendments [15].

18.2 MHRA and Ethical Approval

Prior to the initiation of the study, the clinical trial of an investigational medicinal product (CTIMP) application, the protocol and associated documentation must be approved by the MHRA and given a favourable opinion by an EC. A copy of this written approval and any correspondence with the MHRA and EC will be available at the clinical site and will be provided to the sponsor.

18.3 Administration of Radiation

Dr Stuart Mair will be the ARSAC practitioner for this study, which includes the administration of radiation at Quotient. Administration will be conducted in accordance with Dr Mair's current ARSAC practitioner licence and Quotient's current ARSAC Employer licence. Additionally, a research application will be submitted to ARSAC to obtain approval for the conduct of the study before dosing.

Before submitting to the ARSAC, a summary of available non-clinical tissue distribution and excretion information on [^{14}C]-rencofilstat will be submitted to the Radiation Protection Division of UK Health Security Agency for human dosimetry calculations in order to facilitate the selection of the dose of radioactivity to be administered. The final report from the UK Health Security Agency will be included in the application to the ARSAC.

The protocol will be reviewed and the final version will be approved by the ARSAC practitioner, Dr Stuart Mair.

18.4 Source Data

A study-specific source document identification list will be finalised with the sponsor prior to the start of the clinical phase of the study. The document will identify what data should be considered source data for this study.

For this study, electronic data capture will be used where possible and data will be automatically recorded into an eCRF. In instances where paper source documents are used, data to be transcribed into the eCRF will be identified using a Source Document Identification List, as governed by Quotient's SOPs.

18.5 Declaration of the End of the Study

The end of the study is defined as the last visit of the last subject (eg discharge from the study). Any changes to this definition will be notified as a substantial amendment (see Section 17.1).

The EC and MHRA should be notified of the conclusion of the study within 90 days of the end of the study, or within 15 days if the study is terminated early, clearly explaining the reasons for the termination.

The ARSAC Practitioner will also be notified of the end of trial or early termination of the trial within an appropriate timeframe.

18.6 Document Storage and Archiving

All documentation and correspondence pertaining to the study (source data, raw data, letters etc) will be kept in accordance with the ICH guidelines for GCP 1996, updated 2002, Integrated Addendum E6 (R2) dated 09 Nov 2016 (ICH GCP Section 4.9.5) [20], The Medicines for Human Use (Clinical Trials) Regulations 2004 [21], The Medicines for Human Use (Clinical Trials) Amendment Regulations 2006 [22],[23] and The Medicines for Human Use (Clinical Trials) (Amendment) (EU Exit) Regulations [25].

All study related documents will be retained for a minimum period of 15 years. After this time, the sponsor will be contacted to ascertain whether continued storage or destruction is required in accordance with current regulations.

18.7 Protection of Personal Data and Confidentiality

Personal data are securely stored to prevent unauthorised access, disclosure, dissemination, alteration or loss of information and unauthorised personal data processing. Access to personal information is restricted so that only personnel who are required to access personal data as part of their job role can do so. All personnel who access personal information are bound by a duty of confidentiality.

Technical arrangements surrounding the electronic storage and use of data are as follows:

- Computers storing electronic personal data are protected by antivirus software and the network on which computers are linked are protected by industry grade firewalls
- Off-site personnel can only access networked computers through a virtual private network
- Electronic access of data is limited according to user roles
- All data are stored on password protected computers

Organisational arrangements are as follows:

- All buildings are secured by key-card access
- Manual files of personal data are stored within locked cabinets/restricted areas of the clinical unit that can only be accessed by authorised personnel
- Data security and/or confidentiality provisions are utilised in agreements with third parties
- Documented Back-up and disaster recovery procedures are in place
- Internal audit and compliance functions provide regulatory oversight

The personal data of volunteers will be pseudonymised in that they will only include health, initials, date of birth and demographics (gender and ethnicity) and cannot be linked back to the individual by the recipient. The sponsor shall be the data controller in respect of the personal data of the study subjects collected in connection with the study, and shall act in accordance with the relevant data protection laws in relation to the collection and processing of those personal data. The study subjects' pseudonymised personal data shall be collected and processed for the purposes of the study and may also be added to research databases and used in the future by the sponsor and its affiliates for certain additional clinical research, for product regulation and safety reporting purposes and for ensuring compliance with legal requirements. The study subjects' pseudonymised personal data may be processed for such purposes by other parties including: the sponsor's affiliates and licensing partners, its business partners, regulatory agencies and other health authorities, and ECs. The study subjects' authorisation for such use and disclosure shall be obtained by the study subjects signing the ICF for the study.

Additionally, Quotient personnel are contractually bound by a duty of confidentiality and receive training on this matter.

18.8 Data Security Breach

Quotient has a comprehensive process in place for identifying, assessing, resolving and reporting any potential data security breach. All staff are trained in the identification of potential data security breaches. Potential breaches are managed by appropriately trained quality assurance (QA) personnel in accordance with Quotient's SOPs. After robust assessment of data breaches, those deemed serious will be reported to the sponsor and Information Commissioner's Office, as applicable.

19 Quality Control and Quality Assurance

Quality control of all data collected from this study will be performed in accordance with Quotient's SOPs. This study (or elements thereof) may be subject to Quotient QA audit, in line with current internal auditing procedures. Similarly, the study (or elements thereof) may be subject to sponsor QA audit.

19.1 Monitoring

GCP requires that studies are adequately monitored. The sponsor should determine the appropriate extent and nature of monitoring. A study monitor, independent of Quotient, will be appointed to verify that the study is conducted in accordance with current GCP, regulatory requirements, the protocol and that the data are authentic, accurate and complete.

The investigator agrees to receive visits from a study monitor and provide assistance to verify protocol implementation, source completion and transcription of data into the eCRF, document storage and AE reporting.

Quotient will extend the professional privilege of access to the subjects' clinical source documents to the study monitor, EC, regulatory bodies or other authorised personnel (eg auditor) for the purposes of source data verification.

Following completion of the study both study related documents and subject data may be sent to the sponsor at a location outside of the UK where data protection laws differ. In the interests of confidentiality, subjects will not be identified on any such documents or data, and specific subject consent for such a disposition will be obtained.

20 Finance and Insurance

The sponsor Hepion Pharmaceuticals, Inc has funded this study. A no-fault clinical trials insurance has been obtained by the sponsor. The sponsor insurance will compensate subjects in accordance with the Association of the British Pharmaceutical Industry Guidelines for Phase I Clinical Trials 2018 edition [18].

21 Publication

Please refer to the Master Services Agreement for information on publication.

Quotient shall have the right to publish the results of the research, subject to the sponsor's prior written consent, which shall not be unreasonably withheld or delayed. Following the receipt of such consent, Quotient shall submit a copy of the proposed publication to the sponsor who shall have 30 days in which to request amendments thereto which, to the extent that such proposed amendments are reasonable, Quotient shall be obliged to incorporate prior to such publication.

The sponsor undertakes that, prior to publication of any information, article, paper, report or other material concerning the research, it will submit a copy of such publication to Quotient who shall have 30 days in which to request amendments thereto which, to the extent that such proposed amendment are reasonable, the sponsor shall be obliged to incorporate prior to such publication.

22 References

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[22] The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2006 No. 1928.

[23] The Medicines for Human Use (Clinical Trials) Amendment (No. 2) Regulations. Statutory Instruments 2006 No. 2984.

[24] The Medicines for Human Use (Clinical Trials) Amendment Regulations. Statutory Instruments 2008 No. 941.

[25] The Medicines for Human Use (Clinical Trials) (Amendment) (EU Exit) Regulations. Statutory Instruments 2019 No. 744.

Appendix 1 Clinical Laboratory Parameters

Haematology	Clinical Chemistry	Virology	Urinalysis	Drugs of Abuse
Basophils Eosinophils Haematocrit (Packed Cell Volume- PCV) Haemoglobin Lymphocytes Mean Cell Haemoglobin (MCH) Mean Cell Haemoglobin Concentration (MCHC) Mean Cell Volume (MCV) Monocytes Neutrophils Platelet Count Red Blood Cell (RBC) Count White Blood Cell (WBC) Count	Alanine Aminotransferase (ALT) Albumin Alkaline Phosphatase Aspartate Aminotransferase (AST) Bicarbonate Bilirubin (Total) Bilirubin (Direct; only if Total is elevated) Calcium Chloride Creatine Kinase (CK) Creatinine eGFR will be calculated at screening using CKD-EPI 2009 Gamma Glutamyl Transferase (GGT) Glucose Glucose (Fasting) Potassium Phosphate (Inorganic) Protein (Total) Sodium Urea Serum Cholesterol (fasted) High-Density Lipoprotein (HDL) Low-Density Lipoprotein (LDL) Triglycerides (TRI) Amylase Lipase	Hepatitis B Surface Antigen Hepatitis C Virus Antibody HIV Antibody **If Required:** SARS-CoV-2 Antigen	Bilirubin Blood Glucose Ketones Leukocytes Nitrites pH Protein Specific gravity Urobilinogen **At discretion of investigator based on urinalysis results** Microbiology Urine Microscopy	Amphetamines Barbiturates Benzodiazepines Cocaine Marijuana/Cannabis Methadone Methamphetamine/ Ecstasy Morphine/Opiates Phencyclidine Tricyclic Antidepressants

Appendix 2 Schedule of Assessments

Study day	-28 to -2	-1		1											2	3	4	5	6	7	8
				Times after dosing (h)																	
	S	A[a]	P[a]	0	0.5	1	1.5	2	3	4	6	8	12	16	24	48	72	96	120	144	168
General Assessments																					
Informed Consent	X																				
Medical History	X	X[b]																			
Weight, Height and BMI	X	X[c]																			
Vein Assessment	X																				
Carbon Monoxide Breath Test	X	X																			
Drug Screen	X	X																			
Alcohol Breath Test	X	X																			
SARS-CoV-2 Antigen[d]		X																			
IMP Administration				X																	
Safety Assessments																					
Physical Examination	X																				
Targeted (symptom driven) Physical Examination[e]			X																		
Safety Blood Samples[f]	X		X												X						
eGFR[g]	X																				
Urinalysis	X		X												X						
Single 12-Lead ECGs	X		X			X				X					X						
Vital Signs[h]	X		X			X				X					X						
Adverse Events	←----------X----------→																				
Prior and Concomitant Medication	←----------X----------→																				
Mass Balance, PK and Met Prof and ID Assessments																					
Whole Blood Samples for Rencofilstat			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma Samples for TR			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Whole Blood Samples for TR			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Whole Blood Samples for Met Prof and ID			X					X				X			X	X			X		
Urine Samples for TR and Met Prof and ID[i]			←----------X----------→																		
Faecal Samples for TR and Met Prof and ID[j]		←----------X----------→																			

Appendix 2 Schedule of Assessments (continued)

Study day	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	28 (+2 days)	35 (±2 days)	Home Collections[n]
	Times after dosing (h)																				
	192	216	240	264	288	312	336	360	384	408	432	456	480	504[k]	528[l]	552[l]	576[l]	600[l]	Return Visits[m]		
General Assessments																					
SARS-CoV-2 Antigen[d]																			X	X	
Safety Assessments																					
Targeted (symptom driven) Physical Examination[e]														X[ko]							
Safety Labs[f]														X[ko]							
Urinalysis														X[ko]							
Single 12-Lead ECGs														X[ko]							
Vital Signs[h]														X[ko]					X	X	
Adverse Events	←----------X----------→																				
Prior and Concomitant Medication	←----------X----------→																				
Mass Balance, PK and Met Prof and ID Assessments																					
Whole Blood Samples for Rencofilstat	X	X		X		X		X		X		X		X[p]							
Plasma Samples for TR	X	X		X		X		X		X		X		X[p]							
Whole Blood Samples for TR	X	X		X		X		X		X		X		X[p]							
Whole Blood Samples for Met Prof and ID	X			X		X				X		X									
Urine Samples for TR and Met Prof and ID[i]	←----------X----------→																				
Faecal Samples for TR and Met Prof and ID[j]	←----------X----------→																				

A: admission; BMI: body mass index; ID: identification; Met Prof: Metabolite Profiling; P: pre-dose; PK: pharmacokinetic(s); S: screening; TR: total radioactivity

[a] Eligibility will be re-assessed at admission/pre-dose

[b] Update only

[c] Weight only

[d] Testing for SARS-CoV-2 may be performed based on current infection rates and availability of tests. If required, it is planned that testing will comprise an antigen test performed on Day -1 prior to admission to the clinical unit and at return visits. The decision on COVID-19 testing and the definition of the testing time points are subject to change based on the current risk mitigation in place and will be agreed by the study team and documented in the ISF via the Clinical Kick-Off Meeting minutes

[e] Targeted (symptom driven) physical examination of the relevant body system(s) as clinically indicated, as per the investigator's judgement

[f] Haematology and clinical chemistry at each time point including virology at screening

[g] eGFR will be calculated at screening using the CKD-EPI equation for eligibility purposes

[h] Blood pressure and heart rate will be measured at every time point; oral temperature will be checked at screening, pre-dose and at first return residential visit (Day 28 +2 days) only
[i] Urine will be collected for the following intervals: A single urine sample will be collected at pre-dose (or the first void of the day), then at: 0-12 h, 12-24 h and then for 24 h intervals until mass balance criteria have been met (see Section 8.1 for details of mass balance criteria)
[j] Faeces will be collected at pre-dose (sample will be taken between admission and pre-dose), then at 24 h intervals until mass balance criteria have been met (see Section 8.1 for details of mass balance criteria)
[k] Discharge from clinical unit. Discharge assessments will be performed at the time of actual discharge. It is planned that subjects will remain resident in the clinical unit until 504 h post-dose (Day 22). Subjects will be discharged from the clinical unit on Day 22 provided they have met the following mass balance discharge criteria: mass balance cumulative recovery of >90% OR <1% of the dose administered collected in urine and faeces within 2 separate, consecutive 24 h periods. It is also planned that subjects will be discharged from the clinical unit as a group if all subjects have achieved the mass balance discharge criteria
[l] If mass balance discharge criteria have not been met by all subjects by Day 22, the residency period for the subjects not achieving the mass balance criteria may be extended up to a maximum of 96 h (Day 26). Urine and/or faeces will be collected during the additional residency period
[m] If mass balance discharge criteria have not been met by all subjects by the end of the additional residency (Day 26), subjects not achieving the mass balance criteria may be required to make up to 2 return visits (each of 24 h duration) at (nominally) Day 28 (+2 days) and Day 35 (±2 days) for additional collection of excreta samples. Only urine and/or faeces will be collected during the return visits
[n] If the mass balance criteria are not met following the return visits, then home collections of urine and/or faeces may be requested at the discretion of the investigator for individual subjects
[o] In the event of early withdrawal, all safety discharge assessments should be completed
[p] If the residency period for subjects not achieving the mass balance criteria is extended, a further sample will be taken at actual discharge

Appendix 3 Protocol Amendment Summary of Changes

Protocol Version 3.0

In Protocol Version 2.0 (dated 10 Mar 2023), it was noted that there was a urinalysis sample taken at 4 h post-dose. This sample is not required clinically and would require an aliquot of urine to be removed from the mass balance urine collections at a key time point. The 24 h post-dose urinalysis sample is deemed adequate and clinically appropriate. Therefore, the 4 h post-dose urinalysis sample has been removed from the protocol.

All relevant sections of the protocol have been updated, including:

Document History Page; Appendix 2 Schedule of Assessments.

As these changes do not significantly affect the scientific value of the trial, the safety or physical or mental integrity of the subjects of the trial, or the quality of the IMP, the amendment to the protocol is considered to be non-substantial.

Protocol Version 2.0

The 11 timepoints for the whole blood metabolite ID collections have been rearranged to occur over a longer duration of time to ensure that the study objectives will be met. Pharmaron provided adjustments to the timings of sample collection. The timings but not the number of samples or blood volumes have been updated.

In Protocol Version 1.0 (dated 21 Dec 2022), the time points for whole blood metabolite ID collection were scheduled to be taken at pre-dose, 2, 4, 8, 12, 24, 48, 72, 96 120 and 168 h post-dose. The protocol has been updated to remove samples taken at 4, 12, 72, 96 and 168 h post dose and add samples at 192, 264, 312, 408 and 456 h post-dose. Therefore, the updated metabolite ID time points are as follows: pre-dose, 2, 8, 24, 48, 120,192, 264, 312, 408 and 456 h post-dose.

Additionally, it was noted that in the footnotes of Table 1 and the table ‘Investigational Medicinal Product, Dose and Mode of Administration’ in the synopsis, the IMP was incorrectly stated as a salt form. These footnotes have been removed.

Furthermore, minor typographical corrections have been made.

All relevant sections of the protocol have been updated, including:

Document History Page; Synopsis; 5.1 Introduction; 5.2 Investigational Medicinal Product; Appendix 2 Schedule of Assessments.

As these changes do not significantly affect the scientific value of the trial, the safety or physical or mental integrity of the subjects of the trial, or the quality of the IMP, the amendment to the protocol is considered to be non-substantial.

Signatures for Quotient Sciences

CONFIDENTIALITY AND GCP COMPLIANCE STATEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, and any other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use E6 (R2) Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in Section 16.3 of this protocol.

Information taken from the study protocol may not be disseminated or discussed with a third party without the express consent of the sponsor.

Litza McKenzie MBChB, BScMedSci (Hons)	See electronic signature at the end of the document
Principal Investigator Senior Clinical Research Physician	Signature
	Date

Signatures for Sponsor

Jill A Greytok
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