CLINICAL STUDY PROTOCOL

A PHASE 1 STUDY TO ASSESS THE MASS BALANCE, EXCRETION, AND PHARMACOKINETICS OF [^{14}C]-GBT021601, AN ORAL HEMOGLOBIN S POLYMERIZATION INHIBITOR, IN HEALTHY PARTICIPANTS

CONFIDENTIAL

Sponsor code: GBT021601-013
ICON code: GBT2180A-2180AX
EudraCT number: 2022-003108-34

GBT021601 ADME Microtracer Study

Investigational product:	GBT021601
Clinical phase:	Phase 1 study
Indication to be studied:	Not applicable
Sponsor:	Global Blood Therapeutics, Inc., *a wholly owned subsidiary of Pfizer* 181 Oyster Point Boulevard South San Francisco, CA 94080 United States
Contract Research Organization and Clinical Site:	ICON – Early Development Services Van Swietenlaan 6 9728 NZ Groningen The Netherlands
Principal Investigator:	

Version 3.0, 13 Mar 2023
Version 2.0, 27 Oct 2022
Version 1.0, 29 Sep 2022

This study will be performed in compliance with the principles of Good Clinical Practice.

SERIOUS ADVERSE EVENT CONTACT INFORMATION

In case of a serious adverse event (see Appendix 8.2), the Principal Investigator will send a report within 24 hours of notification to the Sponsor or designee, as per the instructions provided to the site.

SUMMARY OF CHANGES

The following changes have been introduced in this Version 3.0 of the protocol (dated 13 Mar 2023) and are given as a combination of *double underlined and italic text*. Deleted text is given as ~~double strikethrough~~.

The changes were introduced:

- A change in the name of the Principal Investigator.
- A specification of the Sponsor's name and reference to the IB.
- Coronavirus disease 2019 (COVID-19) testing may be done at the discretion of the Investigator at the 24-hour visits.
- Blood collections for metabolite identification analysis were added to the Day 84 visit and beyond.

The changes can be found in:

- Title page, Signature pages, Contact information, and Synopsis
- Table 1, Schedule of Assessments
- Section 1.1.2
- Section 3.5.1.4
- Section 8.2.3

The following changes were introduced in Version 2.0 of the protocol (dated 27 Oct 2022). The changes introduced were requested by the Independent Ethics Committee. In addition, minor typos were corrected and editorial changes were made.

- Additional considerations that justified the choice of the 200 mg dose of GBT021601 have been added to the rationale for the selected dose.
- CCI [redacted]
- Correction of an error in the numbering of the exclusion criteria (Exclusion Criterion 12 was accidentally repeated).

The changes were made found in:

- Section 3.3.1, Inclusion Criteria; Inclusion Criterion 7 and Inclusion Criterion 8
- Section 3.3.2, Exclusion Criteria; Exclusion Criterion 13 through 27
- Section 3.4.4, Selection of Doses in the Study
- Section 3.4.8.2, Contraception
- Section 8.3, Pregnancy
- Section 7, REFERENCES

SYNOPSIS

Study Title

A PHASE 1 STUDY TO ASSESS THE MASS BALANCE, EXCRETION, AND PHARMACOKINETICS OF [^{14}C]-GBT021601, AN ORAL HEMOGLOBIN S POLYMERIZATION INHIBITOR, IN HEALTHY PARTICIPANTS

Short Study Title

GBT021601 ADME Microtracer Study

Study Codes

Sponsor code : GBT021601-013
ICON code : GBT2180A-2180AX
EudraCT number : 2022-003108-34

Sponsor

Global Blood Therapeutics (GBT), Inc., *a wholly owned subsidiary of Pfizer*
181 Oyster Point Boulevard, South San Francisco, CA 94080, US
Sponsor's contact : PPD

Contract Research Organization and Clinical Site

ICON – Early Development Services, Van Swietenlaan 6, 9728 NZ Groningen, The Netherlands

Principal Investigator

PPD

Objectives

Primary : To determine the whole blood and plasma concentrations of [^{14}C]-GBT021601 total radioactivity.
To assess the mass balance by determining [^{14}C]-GBT021601 total radioactivity excreted in urine and feces.
To determine the pharmacokinetics (PK) of GBT021601 in whole blood, plasma, and urine.

Secondary : To assess the safety and tolerability of GBT021601 administration in healthy participants.
To characterize and identify metabolites of [^{14}C]-GBT021601 in whole blood, plasma, urine, and feces.

CCI [redacted]

Design and Treatments

This Phase 1, single-center, open-label study will be conducted to evaluate the absorption, distribution, metabolism, and excretion (ADME) of GBT021601 in 8 to 10 healthy male or female participants. GBT021601 will be administered as a single oral dose of 200 mg GBT021601, containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601, under fasted conditions.

After dosing on Day 1, blood (whole blood and plasma) and excreta (urine and feces) will be collected for up to 7 months due to the long elimination half-life from blood and plasma (approximately 28 to30 days) of GBT021601, depending on how much radioactivity is recovered in excreta and the rate of elimination of radioactivity. Vomitus will be collected, if possible, if a participant vomits within 24 hours after study drug administration. Participants will remain confined in the clinical research unit (CRU) until discharge on Day 29 (ie, 1 half-life period). After discharge from the CRU, participants will return to the CRU for up to 11 visits. Each follow-up visit to collect blood and excreta will be a 24-hour stay in the CRU.

Concentrations of total radioactivity in blood and excreta will be assayed weekly during the 30-day confinement phase and thereafter within 3 days of the 24-hour collections. This will enable the Sponsor/Investigator to determine how long samples should be collected. Criteria for ending the collection of blood and excreta in a participant after Day 29 are: 1) ≥90% of the administered radioactive dose is recovered in excreta collected to date, or 2) radioactivity is undetectable in urine and feces during 2 consecutive a 24-hour collection periods, or 3) until the end-of-study (EoS) visit on Day 206.

Participants will receive the appropriate containers to collect all feces at home within 24 hours prior to admission on Day -1 and each of the 24-hour stays. This sample will be used if no feces is produced between admission on Day -1 and dosing (as blank sample) or during the 24-hour stays after Day 29, respectively.

The total duration of study participation from screening until EoS visit will be up to approximately 234 days.

Study Schedule

Screening	: Between Day -28 and Day -2
Treatment period	: One period in the CRU from Day -1 (admission) to Day 29, with dosing on Day 1. Participants will return to the CRU for up to 11 visits of 24 hours each: on Days 35 and 42 (±1 day for each visit); and on Days 56, 70, 84, 98, 112, 136, 150, 178, and 206 (±2 days for each visit) for the collection of blood, plasma, urine, and feces (depending on whether criteria for ending the collection of blood and excreta are met).
Follow-up	: Follow-up assessments will be performed at EoS (on Day 206, or at a separate visit 7 [±3] days after the last 24-hour visit if criteria for ending the collection of blood and excreta are met before Day 206), or at early termination.

Participants

8 to 10 healthy male or female participants

Main Criteria for Inclusion

Age	: 18 to 55 years, inclusive, at screening
Body mass index	: 18.0 to 27.0 kg/m^2, inclusive, at screening
Body weight	: ≥50 kg, inclusive, at screening

Investigational Product

Active substance	: GBT021601
Activity	: Inhibition of sickle cell hemoglobin polymerization
In development for	: Sickle cell disease
Strength	: 0.5 mg/mL containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601
Dosage form	: Oral solution
Manufacturer	: Pharmacy at ICON

Variables

Safety : Adverse events, clinical laboratory (clinical chemistry, hematology, and urinalysis), vital signs (supine blood pressure and pulse, respiratory rate, and body temperature), and 12-lead electrocardiograms.

Pharmacokinetics : Whole blood and plasma concentrations of [^{14}C]-GBT021601 total radioactivity and GBT021601, and their PK parameters including but not limited to: C_{max}, t_{max}, AUC_{0-t}, AUC_{0-inf}, k_{el} (λ_z), and $t_{1/2}$ (and CL/F and V_z/F for GBT021601 only).
Urine and feces (and, if applicable, vomitus) concentrations of [^{14}C]-GBT021601 total radioactivity and corresponding PK parameters: Ae_{urine}, fe_{urine}, Ae_{feces}, fe_{feces}, (if applicable, in vomitus: Ae_{vomit} and fe_{vomit}), Ae_{total}, and fe_{total}; urine concentrations of GBT021601 and corresponding PK parameters: CL_R, Ae_{urine}, and fe_{urine}.
CCI [redacted] The ratio of whole blood to plasma for total radioactivity CCI [redacted] will also be calculated.
Metabolite identification endpoints, including the structural identification of selected metabolites derived from [^{14}C]-GBT021601 in whole blood and plasma samples, and selected, pooled urine and fecal samples; in collaboration with Sponsor and laboratory personnel (results will be reported separately).

Statistical Methods

Sample size calculation : The sample size is not based on statistical considerations, but on a conventional number of participants that is considered sufficient to achieve the study objectives.

Safety parameters : Descriptive statistics.

PK parameters : Individual whole blood, plasma, urine, and feces PK parameters will be estimated using noncompartmental analysis for total radioactivity and GBT021601, as appropriate.
Descriptive statistics for all relevant PK parameters: n, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation. No formal statistical analyses are planned.

Table 1 Schedule of Assessments

Study Days / Assessments	Screening (-28 to -2)	Long-term Confinement in CRU (Days -1 to 29) [a]													24-hour Visits [a, b]										
		-1	1	2	3	4	5	6	7	8	14	21	28	29	35 -36	42 -43	56 -57	70 -71	84 -85	98 -99	112 -113	136 -137	150 -151	178 -179	206-207 [EoS/ET]
Long-term confinement		X----------												X											
Confinement for 24 hours [a]															X	X	X	X	X	X	X	X	X	X	X
Window for 24-hour visits															±1 day		±2 days								
Informed consent	X																								
Demographics	X																								
Medical and surgical history	X	X [c]																							
Review inclusion/exclusion criteria	X	X																							
Height/body weight/BMI [d]	X	X																							
Vital signs [e]	X		X	X											X				X						X [b]
12-lead ECGs [f]	X	X	X																						
Physical examination [g]	X	X																							X [b]
Pregnancy test (females only) [h]	X	X																X		X		X		X	X [b]
FSH test (females only)	X																								
Clinical safety laboratory [i]	X	X							X				X						X						X [b]
Coagulation panel (PT, aPTT, INR)	X																								
Creatinine clearance [j]	X																								
Serology (hepatitis A/B/C, HIV)	X																								
COVID-19 testing (PCR)		X		X											X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X[r]	X [b, r]
Drug and alcohol screening	X	X													X----------at indication only----------X [b]										
Study drug administration			X																						
[redacted] [cci]			[redacted]																						
Blood sampling for PK of GBT021601 and total radioactivity [l]			X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
CCI [redacted]			[redacted]										[redacted]												
Blood sampling for metabolite identification [n]			X	X	X	X	X	X	X	X	X	X	X						X	X	X	X	X	X	X
Collection of urine [o]			X	X	24-hour collection intervals										X	X	X	X	X	X	X	X	X	X	X
Collection of feces [p]		X	24-hour collection intervals												X	X	X	X	X	X	X	X	X	X	X
Collection of vomitus, if produced [q]			X																						
Previous/concomitant medication	X	X	X----------																						X [b]

Adverse events	X	X	X----------X [b]

Abbreviations: aPTT=activated partial thromboplastin time; BMI=body mass index; COVID-19=coronavirus disease 2019; CRU=clinical research unit; ECG=electrocardiogram; EoS=end of study; ET=early termination; FSH=follicle-stimulating hormone; HIV=human immunodeficiency virus; INR=international normalized ratio; PCR=polymerase chain reaction; PK=pharmacokinetic(s); PT=prothrombin time

a. Participants will be confined in the CRU from Day -1 to 29. Thereafter, participants will return to the CRU for up to 11 visits of 24 hours each to collect whole blood, plasma, urine, and feces. Criteria for ending the collection of blood and excreta in a participant after Day 29 are: 1) ≥90% of the administered radioactive dose is recovered in excreta collected to date, or 2) radioactivity is undetectable in urine and feces during 2 consecutive a 24-hour collection periods, or 3) until the last visit on Day 206.
b. If the criteria for ending the collection of blood and excreta are met after Day 29 and before Day 206, participants will return to the CRU at a separate visit 7 (±3) days after the last 24-hour visit to perform the EoS assessments as indicated with a 'b' in the column for Day 206/207 (EoS/ET).
c. Any updates to medical and surgical history will be recorded.
d. Height will be performed at screening only. BMI will be calculated using the height obtained at screening.
e. Vital signs (heart rate, blood pressure, respiratory rate, and body temperature) will be measured after a participant has rested at least 5 minutes in the supine position. Vital signs will be measured at screening, predose on Day 1, and at 1, 2, 8, 12, and 24 hours postdose, and on Days 35, 84, and 206 (EoS/ET).
f. 12-lead ECGs will be recorded after a participant has rested at least 5 minutes in the supine position. Triplicate ECGs will be recorded at screening only; thereafter, standard single 12-lead ECGs will be taken on Day -1 and on Day 1 at predose and at 1 and 2 hours postdose.
g. A complete physical examination will be conducted at screening. Targeted physical examinations will be conducted at the other time points. Symptom-directed physical examinations may also be conducted at any time, per the Investigator's discretion.
h. For females only: serum pregnancy test is required at screening only; urine pregnancy test at all other visits, with serum confirmation if positive.
i. Clinical safety laboratory (including hematology, serum chemistry, and urinalysis) will be obtained at screening and on Days -1, 7, 28, 84, and 206 (EoS/ET).
j. Creatinine clearance (CL_{cr}) will be calculated at screening using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.
CCI [redacted].
l. Whole blood and plasma samples for PK of GBT021601 and total radioactivity will be taken at predose (within 60 minutes prior to GBT021601 dosing), and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 (Day 2), 48 (Day 3), 72 (Day 4), 96 (Day 5), 144 (Day 7), 168 (Day 8), 312 (Day 14), 480 (Day 21), and 648 hours (Day 28) postdose. Additional samples will be obtained on Days 35, 42, 56, 70, 84, 98, 112, 136, 150, 178, and 206 (depending on if criteria for ending collection of urine and feces are met [see Footnote a], collection of samples may be ended prior to Day 206). Collections up to and including the 8 hours postdose sample will be collected after a participant has rested at least 5 minutes in the supine position. CCI [redacted]
CCI [redacted] n.
Whole blood and plasma samples for metabolite identification will be collected at predose and 1, 2, 8, 12, 24 (Day 2), 48 (Day 3), 72 (Day 4), 96 (Day 5), 120 (Day 6), 144 (Day 7), 168 (Day 8), 312 (Day 14), 480 (Day 21), and 648 hours (Day 28) hours postdose. Collections up to and including the 8 hours postdose sample will be collected after a participant has rested at least 5 minutes in the supine position. *Whole blood and plasma samples for metabolite identification will also be collected at the visits on Days 84 (1992 hours postdose), 98 (2328 hours), 112 (2664 hours), 136 (3240 hours), 150 (3576 hours), 178 (4248 hours), and 206 (4920 hours; EoS/ET).*

o. Urine samples for PK of GBT021601 and total radioactivity will be collected on Day 1 (prior to dosing) and after dosing from 0 to 6 hours, 6 to 12 hours, 12 to 24 hours postdose, and thereafter in 24-hour intervals up to Day 29. Urine will also be collected in 24-hour intervals starting on Days 35 (816 hours postdose), 42 (984 hours), 56 (1320 hours), 70 (1656 hours), 84 (1992 hours), 98 (2328 hours), 112 (2664 hours), 136 (3240 hours), 150 (3576 hours), 178 (4248 hours), and 206 (4920 hours) in accordance with the applicable time windows (depending on if criteria for ending collection of blood and excreta are met [see Footnote a], the collection may be ended prior to Day 206). Selected pooled urine will be used for metabolite identification.

p. Fecal collections for the analysis of total radioactivity will be made at predose on Day -1, and complete collections will be made after dosing in 24-hour intervals up to Day 29. Feces will also be collected in 24-hour intervals starting on Days 35 (816 hours postdose), 42 (984 hours), 56 (1320 hours), 70 (1656 hours), 84 (1992 hours), 98 (2328 hours), 112 (2664 hours), 136 (3240 hours), 150 (3576 hours), 178 (4248 hours), and 206 (4920 hours) in accordance with the applicable time windows (depending on if criteria for ending collection of blood and excreta are met [see Footnote a], the collection may be ended prior to Day 206). Participants will receive the appropriate containers to collect all feces at home within 24 hours prior to admission on Day -1 and each of the 24-hour stays. These fecal samples will be used if no feces is produced between admission on Day -1 and study drug administration (as a blank sample) or during the 24-hour stays after Day 29, respectively. Selected pooled fecal samples will be used for metabolite identification.

q. Collection of vomitus for analysis of total radioactivity concentrations (if possible), if produced between dosing and 24 hours postdose.

r. COVID-19 testing may be performed at the Investigator's discretion.

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LIST OF ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
AMS	accelerator mass spectrometry
BMI	body mass index
CA	competent authority
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CHMP	Committee for Medicinal Products for Human Use
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
COVID-19	coronavirus disease 2019
CRU	clinical research unit
CSP	clinical study protocol
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
ECG	electrocardiogram
eCRF	electronic case report form
EMA	European Medicines Agency
EoS	end of study
ET	early termination
EU	European Union
FDA	Food and Drug Administration
GBT	Global Blood Therapeutics, Inc. (the Sponsor)
GCP	Good Clinical Practice
Hb	hemoglobin
HbS	sickle hemoglobin
HSCT	hematopoietic stem cell transplantation
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
PCR	polymerase chain reaction
PK	pharmacokinetic(s)
QTcF	QT-interval corrected for heart rate using Fridericia's formula
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCD	sickle cell disease
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
US	United States
WMA	World Medical Association
WMO	Wet Medisch-Wetenschappelijk Onderzoek met Mensen (Medical Research Involving Human Subjects Act)

Note: Definitions of pharmacokinetic (PK) parameters are provided in Section 3.5.3.

1. INTRODUCTION

Global Blood Therapeutics (GBT), Inc. (the Sponsor) intends to develop GBT021601, an oral sickle hemoglobin (HbS) polymerization inhibitor, for the treatment of sickle cell disease (SCD).

1.1 Background

1.1.1 Sickle Cell Disease

Sickle cell disease is an inherited disorder caused by a point mutation in the β-globin gene leading to formation of HbS. A primary and obligatory event in the molecular pathogenesis of SCD is the polymerization of intracellular HbS following deoxygenation in the microvasculature. HbS polymerization leads to decreased red blood cell (RBC) deformability, morphologic sickling of RBCs, decreased RBC survival, and microvascular obstruction.[1] Clinically, SCD is a devastating and debilitating disease marked by the pathophysiologic features of hemolytic anemia, vaso-occlusion, and progressive end-organ damage. Despite current standards of care, including hydroxyurea, blood transfusion, and supportive care with analgesia, patients with SCD continue to suffer serious morbidity and premature mortality.

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative therapy for SCD. HSCT in children with SCD is associated with overall and event-free survival rates of 95% and 92%, respectively. However, HSCT use is limited by the paucity of suitable donors, the risk of graft-versus-host disease, infections, infertility, and other long-term transplant-related complications. Moreover, HSCT is generally available only in high-income countries and not commonly used in older patients with significant morbidity.[2]

1.1.2 GBT021601

GBT021601 increases hemoglobin (Hb) oxygen affinity and stabilizes Hb in the oxygenated Hb state, thereby inhibiting polymerization of HbS in RBCs.[3] Proof of concept for this Hb modification approach has been provided with voxelotor, an orally-administered small molecule inhibitor of HbS polymerization developed by ~~GBT~~ *the Sponsor*. Voxelotor was approved by the United States (US) Food and Drug Administration (FDA) in 2019, under the tradename Oxbryta®, for the treatment of SCD in adults and pediatric patients of ≥12 years of age. Also in the European Union (EU), Voxelotor is approved for the treatment of SCD in adults and pediatric patients of ≥12 years of age. In Dec 2021, the FDA approved a supplemental New Drug Application to expand the indicated age range of Oxbryta to include pediatric patients with SCD of ≥4 years in the US.[4] This indication is approved under accelerated approval based on increase in Hb. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trial(s). Voxelotor is an allosteric modifier of Hb-O_2 affinity and following binding to Hb, stabilizes the oxygenated Hb state,[5,6] thereby inhibiting polymerization of HbS in RBCs and in patients with SCD.

GBT021601 shares the same mechanism of action as Oxbryta®. GBT021601 is designed to optimize the potential for clinical benefit derived from stabilizing the oxygenated Hb state as demonstrated by a comparison to voxelotor in a murine model of SCD where GBT021601 was observed to be more potent, have a longer half-life, and achieve greater exposure per dose.[3] Thus, GBT021601 has the potential to achieve a targeted Hb occupancy and attain desired hematological effects at lower doses than voxelotor, therefore reducing pill burden and improving clinical outcomes for individuals living with SCD. CCI [redacted] By addressing this underlying mechanism of SCD, GBT021601 has the potential to be a disease-modifying therapy, leading to improved anemia, reduced hemolysis, and the possibility of reducing the end-organ damage resulting from chronic hemolytic anemia.

1.1.3 Nonclinical Experience

Nonclinical studies have been conducted to characterize GBT021601, including primary and secondary pharmacodynamics, safety, pharmacology, PK, and toxicology. In SCD mice treated with GBT021601, a Hb occupancy of ~6% resulted in a >1 g/dL increase in Hb concentration in all animals. Moreover, at a GBT021601 Hb occupancy of ~29%, GBT021601 caused a sustained and almost complete elimination of circulating sickled RBCs, reduced reticulocyte counts by >50%, increased RBC half-life by 6.1 days, and normalized Hb with a 6.7 g/dL increase from baseline in SCD mice.

1.1.4 Clinical Experience

Two Phase 1 clinical studies (GBT021601-011 and GBT021601-012) in healthy participants are in progress.

A first-in-human single-ascending dose and multiple-ascending dose Phase 1 study in healthy participants is ongoing (GBT021601-011). CCI [redacted].

CCI [redacted]

[redacted]					

[redacted]

CCI [redacted]

A detailed description of the chemistry, pharmacology, efficacy, and safety of GBT021601 is provided in the Investigator's Brochure of GBT021601.[8]

1.2 Risk-benefit Assessment and Risk Mitigation

1.2.1 Risk-benefit Assessment for GBT021601

CCI [redacted]

Based on the clinical and nonclinical experience with GBT021601, pharmacology-based hematological effects are expected to occur with GBT021601 treatment and may include increased RBCs, Hb, hematocrit, and reticulocytes. These hematological effects will be mitigated by monitoring these parameters at select timepoints throughout the study. In addition, participants with concurrent renal impairment (Inclusion Criterion 9) or abnormal hematology/coagulation parameters will be excluded from participation. However, these hematological side effects are not likely to occur with the selected single dose of 200 mg GBT021601 (see Section 3.4.4 for dose selection). CCI [redacted]

There is no expected clinical benefit for the healthy participants who will participate in this study. The information obtained in this study can be used for the further clinical development of GBT021601.

Overall, on the basis of the available nonclinical and clinical data, and prior knowledge, the risk-benefit profile of GBT021601 is considered acceptable for the proposed clinical study.

1.2.2 Risk-benefit Assessment Related to SARS-CoV-2

The risk-benefit assessment for the participants receiving GBT021601 remains unchanged in relation to the coronavirus disease-2019 (COVID-19) pandemic as available clinical and nonclinical results do not suggest that administration of GBT021601 will lead to suppression or modulation of the immune system. In addition, the mode of action does not appear to have a substantial effect on the respiratory or cardiovascular system critically affected by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. As the participants to be included in this study are in general young to middle aged without major comorbidities, the study population is not considered to be a high-risk population for serious COVID-19 disease. Only persons with a negative SARS-CoV-2 test at admission to the clinical research unit (CRU) will be allowed to participate in the study. In addition, all appropriate measures to prevent SARS-CoV-2 infection during the study will be taken as detailed in Section 3.2.1.

1.3 Study Rationale

This study will determine the disposition and metabolism of GBT021601 in humans after oral administration. The use of radiolabeled molecules (usually [^{14}C]) is a common method used to ascertain information on the elimination routes and metabolic fate of a compound at an early stage of development. Blood (whole blood and plasma) and excreta (urine and feces) will be collected for up to 7 months CCI [redacted] depending on how much radioactivity is recovered in excreta and the rate of elimination of radioactivity. The study duration and collection schedule also account for the potential of long-lived GBT021601 metabolites.

The rationale for conducting this study is to 1) identify and quantify levels of circulating metabolites of GBT021601 which may contribute to pharmacology or safety; 2) identify and quantify drug-related materials in excreta which provide the basis for proposed clearance mechanisms that may impact interpatient variability and/or PK of GBT021601 in special populations; and 3) demonstrate that preclinical safety coverage exists for major human metabolites.

2. OBJECTIVES

2.1 Primary

- To determine the whole blood and plasma concentrations of [^{14}C]-GBT021601 total radioactivity.
- To assess the mass balance by determining [^{14}C]-GBT021601 total radioactivity excreted in urine and feces.
- To determine the PK of GBT021601 in whole blood, plasma, and urine.

2.2 Secondary

- To assess the safety and tolerability of GBT021601 administration in healthy participants.
- To characterize and identify metabolites of [^{14}C]-GBT021601 in whole blood, plasma, urine, and feces.

CCI [redacted]

- [redacted]
- [redacted]

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design and Plan

3.1.1 Type of Study

This Phase 1, single-center, open-label study will be conducted to evaluate the absorption, distribution, metabolism, and excretion (ADME) of GBT021601 in 8 to 10 healthy male or female participants. GBT021601 will be administered as a single oral dose of 200 mg GBT021601, containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601, under fasted conditions. For more details on the study drug, see Section 3.4.1.

After dosing on Day 1, blood (whole blood and plasma) and excreta (urine and feces) will be collected for up to 7 months, depending on how much radioactivity is recovered in excreta and the rate of elimination of radioactivity. Vomitus will be collected, if possible, if a participant vomits within 24 hours after study drug administration. Participants will remain confined in the CRU until discharge on Day 29. After discharge from the CRU, participants will return to the CRU for up to 11 visits. Each follow-up visit to collect blood and excreta will be a 24-hour stay in the CRU.

Concentrations of total radioactivity in blood and excreta will be assayed weekly during the 30-day confinement phase and thereafter within 3 days of the 24-hour collections. This will enable the Sponsor/Investigator to determine how long samples should be collected. Criteria for ending the collection of blood and excreta in a participant after Day 29 are: 1) ≥90% of the administered radioactive dose is recovered in excreta collected to date, or 2) radioactivity is undetectable in urine and feces during 2 consecutive a 24-hour collection periods, or 3) until the end-of-study (EoS) visit on Day 206.

Participants will receive the appropriate containers to collect all feces at home within 24 hours prior to admission on Day -1 and each of the 24-hour stays. This sample will be used if no feces is produced between admission on Day -1 and dosing (as blank sample) or during the 24-hour stays after Day 29, respectively.

The total duration of study participation from screening until EoS visit will be up to approximately 234 days. For an overview of the study design, see Table 3.

Table 3 Schematic Study Design

Visit Activity	Screening	Long-term confinement in CRU			24-hour visits (up to 11 visits) [a]
	Eligibility	Admission	Dosing	Assessments/ PK sampling	PK sampling (collection of blood and excreta)
Study Day(s)	-28 to -2	Day -1	Day 1	Days 1 to 29 (ie, discharge)	35-36 (±1 day), 42-43 (±1 day), 56-57(±2 days), 70-71 (±2 days), 84-85 (±2 days), 98-99 (±2 days), 112-113 (±2 days), 136-137 (±2 days), 150-151 (±2 days), 178-179 (±2 days), 206-207 (±2 days; EoS/ET)

CRU=clinical research unit; EoS=end of study; PK=pharmacokinetic(s)

[a] Criteria for ending the collection of blood and excreta in a participant after Day 29 are: 1) ≥90% of the administered radioactive dose is recovered in excreta collected to date, or 2) radioactivity is undetectable in urine and feces during 2 consecutive a 24-hour collection periods, or 3) until the EoS visit on Day 206. If these criteria are met after Day 29 and before Day 206, participants will return to the CRU for EoS assessments at a separate visit 7 (±3) days after the last 24-hour visit.

3.1.2 Screening Period

Participants will report to the medical screening facility or clinical site for the eligibility screening (see Section 3.3 for inclusion and exclusion criteria) within 28 days (4 weeks) prior to study drug administration.

Participants will sign the study-specific informed consent form (ICF) prior to any study-specific screening procedures being performed. The written informed consent will be obtained for all participants, regardless of their eligibility for the study. The signed ICFs will be retained and archived at ICON and a copy will be provided to the participant.

Eligibility screening will consist of the assessments as presented in the schedule of assessments (Table 1).

3.1.3 Treatment and Follow-up Period

Participants will be in the CRU for 1 treatment period. Participants will be admitted to the CRU on Day -1, which is the day prior to Day 1, the day of study drug administration. Participants will be discharged on Day 29, after completion of assessments. After discharge, participants will return to the CRU for up to 11 visits (depending on whether criteria for ending the collection of blood and excreta are met) of 24 hours each: on Days 35 and 42 (±1 day for each visit), and on Days 56, 70, 84, 98, 112, 136, 150, 178, and 206 (±2 days for each visit) to collect whole blood, plasma, urine, and feces. Vomitus will be collected, if possible, if a participant vomits within 24 hours after study drug administration.

Follow-up assessments will be performed at EoS (on Day 206, or at a separate visit 7 [±3] days after the last 24-hour visit if criteria for ending the collection of blood and excreta are met before Day 206). In case of early discontinuation from the study, EoS assessments will be performed at early termination (ET).

Assessments during the treatment period and at the EoS/ET visit will be performed as presented in the schedule of assessments (Table 1).

3.2 Discussion of Study Design

The current design is commonly used for ADME studies. Since the present study is a descriptive study aimed at assessing excretion routes of GBT021601 by evaluating mass balance, there is no study hypothesis, thus a formal statistical sample size calculation is not needed. A sample size of 8 to 10 participants is a commonly accepted number of participants for ADME studies and is considered sufficient to achieve the study objectives (see Section 3.6.3).

CCI [redacted] Subjects will be confined in the CRU for 1 half-life period (until Day 29). To achieve the necessary analytical sensitivity, accelerator mass spectrometry (AMS) will be used to determine total radioactivity in urine, feces, whole blood, and plasma samples (see Section 3.5.4). AMS will permit detection of tracer levels of radioactivity over a long time period after GBT021601 administration, therefore enabling outpatient treatment during the study, which greatly reduces the burden on participating participants.

Healthy participants have been chosen as the study population due to the study design and the low risk of clinically significant toxicity at anticipated exposure levels. Additionally, the magnitude and duration of exposure is sufficiently limited to not be able to provide clear therapeutic benefit and justify patients discontinuing current therapies. Moreover, use of healthy participants as opposed to patients will allow a clearer interpretation of the study results, as there will be no confounding factors resulting from changes in disease state and/or concomitant medications.

For a rationale of the selected dose of GBT021601, see Section 3.4.4. The limited radiation burden by the use of a microtracer dose of ~74 kBq (~2 µCi) of [^{14}C]-GBT021601 allows for inclusion of women of childbearing potential.

3.2.1 COVID-19 Risk Mitigation

This study will be conducted in accordance with guidance from the Central Committee on Research Involving Human Subjects (CCMO [Centrale Commissie Mensgebonden Onderzoek] Dutch competent authority [CA]) on conducting Phase 1 trials in clinical research centers in The Netherlands during the COVID-19 pandemic.

During the entire study, the CRU will implement all recommendations issued by the Dutch government, including specific guidelines related to clinical research executed in CRUs with respect to minimizing the risk of disease spreading. Details on specific procedures are described in the site-specific manual.

In cases where participants are not able to attend study visits due to an infection with SARS-CoV-2, the Investigator will discuss with the Sponsor potential mitigation approaches (eg, extending the visit window). The rationale (eg, the specific limitation imposed by the SARS-CoV-2 infection that led to the inability to perform the protocol•specified assessment) and outcome of the discussion will be documented in the electronic case report form (eCRF).

In addition, the following containment measures will be taken during the study:

- Polymerase chain reaction (PCR) testing for SARS-CoV-2 will be performed at the time points indicated in the schedule of assessments (Table 1).
- A participant should not be admitted if there was any close contact with a person who tested positive for SARS-CoV-2 or a COVID•19 patient within the last 2 weeks prior to admission to the clinical research center, or consistent with current policies of the CRU.
- If a participant is tested to be SARS-CoV-2 positive at admission on Day -1, the participant will be excluded from participation with reference to Exclusion Criterion 24, and if needed referred for treatment.
- Physical examinations will be limited.
- If a participant becomes ill and/or is tested to be SARS-CoV-2 positive after dosing, study assessments may be stopped or interrupted at the discretion of the Investigator and in consultation with the Sponsor (see also Section 3.3.4).

The COVID-19 risk mitigation measures will be kept in place for as long as the pandemic is ongoing, as defined by country and site-specific regulations. In the future, SARS-CoV-2 testing may be omitted at the discretion of the Investigator.

3.3 Selection of Study Population

A total of 8 to 10 healthy male or female participants are planned to be included in the study.

CCI [redacted] Therefore, every effort will be made to include as many healthy participants of African descent (with priority given to sub-Saharan Africa) descent as possible.

3.3.1 Inclusion Criteria

Note: Restrictions that apply to the period after admission to the CRU are described in Section 3.4.8, except when they concern a statement of willingness.

The following inclusion criteria must be met for a participant to be eligible for inclusion in the study:

1. Sex : Male or female.
2. Age : 18 to 55 years, inclusive, at screening.
3. Body mass index (BMI) : 18.0 to 27.0 kg/m^2, inclusive, at screening.
4. Body weight : ≥50 kg at screening.
5. Good physical and mental health on the basis of medical and surgical history, physical examination, clinical laboratory (including clinical chemistry, hematology, urinalysis, and coagulation), 12-lead electrocardiogram (ECG), and vital signs, as judged by the Investigator.
6. Females must be nonlactating and nonpregnant (as confirmed by a negative serum pregnancy test at screening and admission for all females), or of nonchildbearing potential (ie, either surgically sterilized or physiologically incapable of becoming pregnant, or at least 1 year postmenopausal [defined as at least 12 months no menses, and confirmed by a follicle-stimulating hormone test, at screening]).
7. Female participants of childbearing potential who have a fertile male sexual partner must agree to use adequate contraception from at least 4 weeks prior to dosing (Day 1) CCI [redacted]. Adequate contraception is defined as using hormonal contraceptives or an intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm, a cervical cap, or a condom. Total abstinence from heterosexual intercourse, in accordance with the lifestyle of the participant, is also acceptable.
8. Male participants, if not surgically sterilized, must agree not to donate sperm, and to use adequate contraception when sexually active with a female partner of childbearing potential, from admission to the CRU CCI [redacted]. Males who are not surgically sterile and sexually active with a pregnant partner must agree to use a condom during this period. Adequate contraception for the male participant and his female partner is defined as using hormonal contraceptives or an intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm, a cervical cap, or a condom. Total abstinence from heterosexual intercourse, in accordance with the lifestyle of the participant, is also acceptable.
9. Creatinine clearance (glomerular filtration rate) as estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula: ≥90 mL/min, at screening.
10. Nonsmoker, defined as having abstained from habitual use of tobacco- or nicotine-containing products (eg, cigarettes, cigars, chewing tobacco, snuff, nicotine patches, and electronic cigarettes) within 6 months prior to screening, who is willing and able to abstain from tobacco/nicotine use during participation in the study.
11. After being informed of the nature and risks of the study, participants must be willing and able to sign the ICF.

3.3.2 Exclusion Criteria

Note: Restrictions that apply to the period after admission to the CRU are described in Section 3.4.8, except when they concern a statement of willingness.

A participant who meets any of the following exclusion criteria will not be eligible for inclusion in the study:

1. Employee of ICON or the Sponsor.
2. History or presence of clinically significant allergic diseases (except for untreated, asymptomatic, seasonal allergies at time of dosing), in the opinion of the Investigator.
3. History or presence of conditions which, in the opinion of the Investigator, are known to interfere with the ADME of drugs, such as previous surgery on the gastrointestinal tract (including removal of parts of the stomach, bowel, liver, gall bladder, or pancreas). Participants who have a history of appendectomy are eligible for enrollment.
4. History of chronic constipation, or recent complaints of an irregular defecation pattern (ie, less than once per day on average) in the opinion of the Investigator.
5. History of surgery requiring general anesthesia (not including local procedures) or major trauma within 12 weeks of screening, or a planned surgery during participation in the study.
6. Significant and/or acute illness at screening or within 5 days prior to study drug administration that may impact safety assessments, in the opinion of the Investigator.
7. Known personal or family history of congenital long QT syndrome or known family history of sudden death.
8. Abnormal ECGs collected at screening or admission, including a QT-interval corrected for heart rate using Fridericia's formula (QTcF) of >450 msec for males and >470 msec for females, or any cardiac rhythm other than sinus rhythm that is interpreted by the Investigator to be clinically significant. A single repeat set of triplicate measurements of a 12-lead ECG may be performed to re-evaluate any ECG abnormalities (ie, to confirm that a participant is eligible).
9. Resting bradycardia or tachycardia (heart rate of <45 or >100 beats per minute, respectively) at screening or admission, based on vital signs measurements. A single repeat measurement may be performed to re-evaluate vital signs abnormalities (ie, to confirm that a participant is eligible). Each of the readings must be not clinically significant to qualify for enrollment into the study.
10. Hypertension, defined as resting (supine) systolic blood pressure of >140 mmHg or resting diastolic blood pressure of >90 mmHg at screening or admission. A single repeat measurement may be performed to re-evaluate vital signs abnormalities (ie, to confirm that a participant is eligible). Each of the readings must be not clinically significant to qualify for enrollment into the study.
11. History of drug addiction (including soft drugs like cannabis products) or alcohol abuse within 2 years prior to the date of consent. Excess alcohol is defined as more than 14 units/week for women and 21 units/week for men. One unit of alcohol is defined as 360 mL of beer, 150 mL of wine, or 45 mL of spirits.

12. Unwillingness or inability to abstain from alcohol within 48 hours prior to screening and each admission (including the 24-hour stays), and during confinement in the CRU.
13. Use of prescription medications within 14 days or 5 half-lives, whichever is longer, prior to dosing (Day 1). An exception is made for hormonal contraceptives, which may be used throughout the study.
14. Use of over-the-counter medication (including vitamin preparations, dietary supplements, or herbal preparations such as St. John's wort) within 7 days prior to dosing (Day 1), or requiring continued use during study participation. Occasional use of acetaminophen/paracetamol (eg, up to 2 grams per day) is permitted during this period and throughout the study.
15. Received a live vaccine within 28 days prior to dosing, or received any other vaccine within 7 days prior to dosing.
16. Consumption of more than 600 mg of caffeine (approximately 6 cups of coffee) per day within 30 days of screening and admission, or unwillingness to abstain from consumption of methylxanthine-containing products (eg, coffee, tea, cola, chocolate, or energy drinks) from 48 hours prior to dosing (Day 1) and during confinement in the CRU.
17. Consumption of grapefruit, grapefruit juice, star fruit, and/or Seville oranges within 14 days prior to admission on Day -1, at the discretion of the Investigator. Unwillingness to abstain from consumption of grapefruit, grapefruit juice, and/or Seville oranges during confinement in the CRU.
18. Unwillingness to abstain from any strenuous physical exercise (such as weight training or aerobics) from 72 hours prior to admission, Day 84, and the EoS visit, and during confinement in the CRU.
19. Participation in a drug study within 30 days prior to screening in the current study, or currently participating in another trial with an investigational drug. Participation in 4 or more other drug studies in the 12 months prior to screening in the current study.
20. Participation in another ADME study with a radiation burden >0.1 mSv in the period of 1 year prior to screening.
21. Donation or loss of more than 470 mL of blood within 90 days prior to admission on Day -1, or donation of plasma/platelets within 2 weeks prior to admission on Day -1. Donation or loss of more than 1.5 liters of blood (for male participants)/more than 1.0 liters of blood (for female participants) in the 10 months prior to admission on Day -1 in the current study.
22. Positive result for drugs (opiates, methadone, cocaine, amphetamines [including ecstasy], cannabinoids, barbiturates, and benzodiazepines), alcohol, and cotinine at screening or admission to the CRU.
23. Positive screen for hepatitis A virus antibody, hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus 1 and 2 antibodies.
24. Positive nasopharyngeal PCR test for SARS-CoV-2 at admission on Day -1.
25. Not fully vaccinated against COVID-19 (ie, vaccinated with at least 2 messenger ribonucleic acid [mRNA] vaccines or 1 viral vector vaccine [booster vaccinations are not required]) or documented recovery from COVID-19 infection.

26. Poor venous access as determined by the Investigator or study staff.
27. Any other condition or prior therapy that, in the Investigator's opinion, would confound or interfere with the evaluation of safety, tolerability, or PK of the study drug, interfere with study compliance, or preclude informed consent.

Please note that participants should refrain from consumption of any foods containing poppy seeds within 48 hours (2 days) prior to screening and each admission to the CRU (including the 24-hour stays) to avoid false positive drug screen results (see Section 3.4.8).

3.3.3 Participant Rescreening

Participants who are discontinued from the study prior to study drug administration may be rescreened as long as the participant was not discontinued from the study due to noncompliance with the protocol (ie, positive urine drugs of abuse screen, etc). If the participant is rescreened, the participant must be reconsented and a new screening number must be used.

3.3.4 Removal of Participants from Assessment

Participation in the study is strictly voluntary. A participant has the right to withdraw from the study at any time for any reason.

The Investigator has the right to terminate participation of a participant for any of the following reasons: difficulties in obtaining blood samples, violation of the protocol, severe AEs or serious adverse events (SAEs), or for any other reason relating to the participant's safety or integrity of the study data.

If a participant is withdrawn from the study, the Sponsor or designee will be informed immediately. If there is a medical reason for withdrawal, the participant will remain under the supervision of the Investigator until satisfactory health has returned.

After signing informed consent, participants who discontinue from the study, drop out, or withdraw for any reason without completing all screening evaluations successfully as well as participants who drop out or withdraw prior to administration of the study drug will be considered screening failures.

A participant who is withdrawn or voluntarily withdraws from the study for any reason, whether related to the study drug or not, after having received the study drug, will be considered an ET participant. If a participant is withdrawn for a reason related to the study drug, according to the judgment of the Investigator, the ET participant will not be replaced. If a participant does not complete the study for a reason not related to the study drug, the ET participant may be replaced after mutual agreement between the Sponsor and the Investigator. The decision regarding the replacement of participants will be documented.

ICON will make every effort to ensure that ET participants who have received study drug complete the ET assessments.

If a participant tests positive for SARS-CoV-2 after dosing, study assessments may be stopped or interrupted at the discretion of the Investigator and in consultation with the Sponsor. During confinement, the participant may be isolated from other study participants and referred for treatment, if necessary. The participant may be followed up in quarantine in the CRU or at home according to guidelines of the Dutch government.

3.3.5 Early Termination of Study

The Sponsor has the right to terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following: 1) the incidence or severity of AEs in this or other studies indicating a potential health hazard to participants; and 2) in the Sponsor's judgment, there are no further benefits to be achieved from the study.

If the study is terminated early, the Sponsor shall inform the Investigator, regulatory authorities, and the Independent Ethics Committee (IEC).

3.4 Treatments

3.4.1 Treatments Administered

On Day 1, a single 200 mg dose of GBT021601 containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601 will be administered as oral solution of 400 mL following an overnight fast of at least 10 hours. In order to rinse the bottle after dosing, the participant will drink 50 mL of tap water (at room temperature) twice. Participants will remain fasting for at least 4 hours after dosing (see Section 3.4.5).

3.4.2 Identity of Investigational Product

Active substance	: GBT021601
Activity	: Inhibition of HbS polymerization
In development for	: Sickle cell disease
Strength	: 0.5 mg/mL containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601
Dosage form	: Oral solution
Manufacturer	: Pharmacy at ICON

The investigational medicinal drug product will be manufactured as a ready-to-administer single-dose bottle containing an oral solution of 400 mL. Details on study drug preparation by the ICON Pharmacy will be described in the Pharmacy Manual and Manufacturing Batch Record, which will be prepared by the ICON Pharmacy.

For details concerning drug storage and drug accountability see Appendix 8.1.

3.4.3 Method of Assigning Participants to Treatment Groups

After obtaining informed consent, participants will receive a screening number and will be screened according to the inclusion and exclusion criteria under provision of an informed consent. Participants who have met all eligibility criteria will receive a participant number upon enrollment in the study (Participant Numbers 101-110). They will receive the participant number just prior to dosing. The participant number will ensure identification throughout the study after study drug administration.

Replacement participants will receive the number of the participant to be replaced, increased by 100 (eg, 201 replacement number for Participant Number 101), and will be administered the same treatment.

Screening failures (defined in Section 3.3.4) and any participants who are eligible for inclusion in the study but do not receive the study drug, will not receive a participant number, and the data obtained will not be recorded in the eCRFs (see Section 5.2.2). For rescreened participants, see Section 3.3.3.

3.4.4 Selection of Doses in the Study

A single 200 mg oral dose of GBT021601 containing ~74 kBq (~2 µCi) of [^{14}C]-GBT021601 has been selected to be administered in this study. This dose level has an acceptable safety and tolerability profile in healthy participants (Study GBT021601-011; see Investigator's Brochure for further details)[8] and is within the anticipated therapeutic range for treatment of SCD.

CCI [redacted] Per FDA guidance (May 2022),[9] the current ADME study should use a dose within the PK linearity range.

A secondary objective of the study is to identify metabolites of ^{14}C-GBT021601 in whole blood, plasma, urine, and feces. The 200 mg dose level of GBT021601 (containing a microtracer amount of ^{14}C-labeled GBT021601) is considered sufficient and necessary to allow for successful GBT021601 metabolite identification. To achieve the necessary analytical sensitivity, both AMS and liquid chromatography will be used for metabolite identification. Although this approach will measure total radioactivity and ^{14}C-labeled metabolites, the presence of nonradiolabeled GBT021601 from the 200 mg dose will be essential to provide sufficient resolution and mass-to-charge of ^{14}C/^{12}C to identify individual metabolites.

3.4.5 Timing of Doses in the Study

On Day 1, the study drug will be administered as an oral solution under supervision of the Investigator or authorized designee with the participant in the upright position. Study drug will be administered to participants between 08:00 and 11:00 hours in the morning after an overnight fast of at least 10 hours. A light supper will be consumed on the evening before dosing (at least 10 hours before dosing). Fasting will continue for a period of at least 4 hours after drug administration (ie, until scheduled lunch).

During fasting, no fluids are allowed except water; however, water is not allowed from 2 hours predose until 1 hour postdose (apart from the water taken with the dose as described in Section 3.4.1). When not fasting, noncaffeinated fluids are allowed ad libitum.

3.4.6 Meals During the Study

A fasting period of at least 4 hours is required before obtaining clinical laboratory blood samples at all time points.

With the fasting requirements as described in Section 3.4.5 and the restrictions with respect to beverages or food as described in Section 3.4.8.3, there are no special requirements related to food and beverage intake. When not fasting, meals and snacks (such as decaffeinated coffee, herbal tea, fruit, biscuits) will be provided according to the clinical site standard operating procedures (SOPs). A light supper will be provided on the evening before dosing when fasting is required until lunch time.

3.4.7 Blinding

This is an open-label study.

3.4.8 Concomitant Medications and Other Restrictions During the Study

Note: Restrictions that apply to the period before admission to the CRU are described in Section 3.3.1 (inclusion criteria) and Section 3.3.2 (exclusion criteria).

3.4.8.1 Concomitant Medications

The use of all prescribed medication is not allowed from 14 days or 5 half-lives, whichever is longer, prior to dosing (Day 1) until the EoS visit. An exception is made for hormonal contraceptives, which are allowed throughout the study. The use of all over-the-counter medication (vitamin preparations, dietary supplements, or herbal medications such as St. John's wort) is not allowed from 7 days prior to dosing (Day 1) until the EoS visit. An exception is made for paracetamol: from admission onwards, the Investigator may permit a limited amount (eg, up to 2 grams per day) of paracetamol for the treatment of headache or any other pain. Other medication to treat AEs may only be prescribed if deemed necessary by the Investigator. If medication is used, the name of the drug, the dose, and dosage regimen will be recorded in the eCRF.

Receipt of a live vaccine is not allowed within 28 days prior to dosing, and receipt of any other vaccine is not allowed within 7 days prior to dosing.

3.4.8.2 Contraception

Male participants, if not surgically sterilized (eg, vasectomy and azoospermia), are required to use adequate contraception (see description below) and not donate sperm from admission to the CRU [redacted].

Female participants of childbearing potential who have a fertile male sexual partner, are required to use adequate contraception (see description below) from at least 4 weeks prior to dosing (Day 1) [redacted]

Adequate contraception is defined as using hormonal contraceptives or an intrauterine device combined with at least 1 of the following forms of contraception: a diaphragm, a cervical cap, or a condom. Total abstinence from heterosexual intercourse, in accordance with the lifestyle of the participant, is also acceptable.

3.4.8.3 Other Restrictions During the Study

The use of methylxanthine-containing beverages or food (coffee, tea, cola, chocolate, or energy drinks) is not allowed from 48 hours prior to dosing (Day 1) and during confinement in the CRU.

Consumption of grapefruit, grapefruit juice, star fruit, and/or Seville oranges is not allowed from 14 days prior to admission on Day -1 and during confinement in the CRU.

Participants should not consume any foods containing poppy seeds within 48 hours (2 days) prior to each admission to the CRU (including the 24-hour stays) as this could cause a false positive drug screen result.

The use of alcohol is not allowed from 48 hours prior to each admission (including the 24-hour stays) and during confinement in the CRU. The use of drugs of abuse and tobacco and nicotine-containing products is not allowed throughout participation in the study.

Strenuous physical exercise (such as weight training or aerobics) is not allowed within 72 hours (3 days) prior to admission (Day -1), Day 84, and the EoS visit, and during confinement in the CRU, as this could result in abnormal clinical laboratory values. Participants are permitted to exercise after the screening visit until 72 hours prior to admission on Day -1.

Participants are not allowed to donate blood during the study until the EoS visit (other than the blood sampling planned for this study).

3.4.9 Treatment Compliance

To ensure treatment compliance, study drug will be administered in the CRU under supervision of the Investigator or authorized designee. Compliance will be further confirmed by bioanalytical assessment of GBT021601 in whole blood, plasma, urine, and fecal samples (see Section 3.5.4).

The exact time of study drug administration will be recorded in the eCRF. The Investigator or authorized designee will examine the participants to ensure all study drug has been swallowed.

3.5 Pharmacokinetic and Safety Measurements and Variables

3.5.1 Pharmacokinetic and Safety Measurements Assessed and Schedule of Assessments

A schedule of assessments is presented in Table 1.

3.5.1.1 Pharmacokinetic Measurements

Details on sample processing, storage, and shipping of blood, urine, and fecal (and vomitus, if produced) samples will be described in the laboratory manual prepared by ICON.

3.5.1.1.1 Blood Sampling

At the time points defined in the schedule of assessments (Table 1), blood samples will be taken for the analysis of GBT021601, total radioactivity, and metabolite identification. Blood samples collected up to and including 8 hours postdose will be obtained after the participant has rested at least 5 minutes in the supine position.

The blood samples will be taken via an indwelling intravenous catheter or by direct venipuncture. The exact times of blood sampling will be recorded in the eCRF.

3.5.1.1.2 Urine Collection

During the intervals defined in the schedule of assessments (Table 1), urine will be collected in urine containers for the analysis of GBT021601 and total radioactivity. Selected pooled urine samples will be used for metabolite identification.

The participants will be instructed to empty their bladders completely before study drug administration and at the end of each collection interval. A blank urine sample will be collected within 12 hours prior to study drug administration. The exact start and end times of urine collection and the urine weight of the entire interval (before and after addition of any urine stabilizers or additives) will be recorded in the eCRF; however the time of each void within the interval may not be recorded.

3.5.1.1.3 Feces Collection

During the intervals defined in the schedule of assessments (Table 1), feces will be collected for the analysis of total radioactivity. Selected pooled fecal samples will be used for metabolite identification.

Blank fecal sample(s) will be collected within 24 hours prior to admission (ie, 48 hours prior to study drug administration). The exact times of fecal collections and the fecal weight of the entire interval will be recorded in the eCRF.

3.5.1.1.4 Vomitus Collection

If a participant vomits between dosing and 24 hours after administration of the study drug, the vomitus should be collected, if possible. Any collected vomitus will be included in the total radioactivity measurements and in the determination of mass balance.

The exact times and weight of the vomitus produced will be recorded in the eCRF.

3.5.1.2 Safety and Tolerability Measurements

Safety and tolerability assessments will consist of AEs, clinical laboratory, vital signs, 12-lead ECGs, and physical examination. Assessments will be performed in accordance with the schedule of assessments (Table 1).

3.5.1.2.1 Adverse Events

Adverse events will be recorded from signing the ICF until completion of the EoS/ET visit. Any clinically significant observations, as determined by the Investigator, in results of clinical laboratory, 12-lead ECGs, vital signs, or physical examinations will be recorded as AEs.

A TEAE is defined as any event not present prior to administration of the study drug or any event already present that worsens in either severity or frequency following exposure to the study drug.

An AE that occurs prior to administration of the study drug will be considered a pretreatment AE.

At several time points before and after drug administration, participants will be asked nonleading questions to determine the occurrence of AEs. Participants will be asked in general terms about any AEs at regular intervals during the study. In addition, all AEs reported spontaneously during the course of the study will be recorded. All answers will be interpreted by the Investigator using the most recent version of the MedDRA (ie, Medical Dictionary for Regulatory Activities) for AEs and will be recorded in the eCRF as reported terms.

The Investigator is responsible for evaluating all AEs, obtaining supporting documents, and ensuring that documentation of the event is complete. Details of each reported AE must include, at minimum, severity, relationship to study drug, duration, and outcome. All serious and non-serious AEs must be followed up until they are resolved or stabilized, or until reasonable attempts to determine resolution of the event are exhausted.

The severity of AEs will be graded using the most recent version of the Common Terminology Criteria for Adverse Events (CTCAE; see Section 8.2.1) 5-point scale: mild (Grade 1), moderate (Grade 2), severe (Grade 3), life-threatening (Grade 4), or death (Grade 5). The relationship between the AEs and the study drug will be indicated as related or not related. Details on rating the severity of AEs and relationship to study treatment are given in Appendix 8.2.

Pregnancy of female participants and female partners of male participants will be monitored along with follow-up, if warranted (see Appendix 8.3).

3.5.1.2.2 Clinical Laboratory

Blood and urine samples for clinical laboratory assessments will be collected, analyzed, and processed according to the clinical site's SOPs. The following parameters will be measured:

- Clinical chemistry (serum quantitatively):
  alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bicarbonate, bilirubin (total, direct, and indirect), blood urea nitrogen, calcium, chloride, creatinine, gamma glutamyl transferase, glucose, lactate dehydrogenase, magnesium, phosphorus, potassium, sodium, total protein, and uric acid.
  At screening only: creatine phosphokinase and creatinine clearance (glomerular filtration rate, estimated by CKD-EPI).
- Hematology (blood quantitatively):
  leukocytes, erythrocytes, hematocrit, Hb, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count (ie, thrombocytes), RBC count, and white blood cell count, with absolute and percent partial automated differentiation (basophils, eosinophils, lymphocytes, monocytes, and neutrophils). Manual differential white blood cell count differentiation will only be performed if there is an abnormality in the blood cell count in accordance with the clinical site's SOPs.
- Urinalysis (urine qualitatively):
  determination of pH and specific gravity, and presence of bilirubin, blood, glucose, ketones, nitrite, protein, leukocytes, and urobilinogen. Urine sediment examinations will only be performed if there is an abnormality in urinalysis in accordance with the clinical site's SOPs.
- Coagulation (blood quantitatively; at screening only):
  prothrombin time, activated partial thromboplastin time, and international normalized ratio.
- Serology (at screening only):
  hepatitis A virus antibody (total), hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus 1 and 2 antibodies.
- Drug and alcohol screen (urine qualitatively):
  opiates, methadone, cocaine, amphetamines (including methylamphetamines and ecstasy), cannabinoids, barbiturates, benzodiazepines, nicotine metabolites (cotinine), and alcohol.
- Pregnancy test (females only):
  β-human chorionic gonadotropin in serum or urine. If a urine pregnancy test is positive, the result must be confirmed by serum pregnancy test immediately.
  At screening only: follicle-stimulating hormone.

Nasal and throat mucosal cell samples will be collected according to the clinical site work instructions. The samples will be tested for SARS-CoV-2 virus using PCR tests as indicated in the schedule of assessments (Table 1), if required by local regulation and guidelines.

In case of unexplained or unexpected clinical laboratory test values, the tests will be repeated as soon as possible and followed up until the results have returned to the normal range and/or an adequate explanation for the abnormality is found. The clinical laboratory will clearly mark all laboratory test values that are outside the normal range, and the Investigator will indicate which of these deviations are clinically significant. Clinically significant laboratory result deviations will be recorded as AEs, and the relationship to the treatment will be indicated (see also Appendix 8.2).

3.5.1.2.3 Vital Signs

Systolic and diastolic blood pressure and pulse will be recorded after the participant has been resting for at least 5 minutes in the supine position. These assessments will be made using an automated device whenever possible. Body temperature and respiratory rate will also be measured.

3.5.1.2.4 Electrocardiogram

A standard 12-lead ECG will be recorded (in triplicate at screening, and as single ECGs thereafter) after the participant has been resting for at least 5 minutes in the supine position. The ECG will be recorded using an ECG machine equipped with computer-based interval measurements. The following ECG parameters will be recorded: heart rate, PR-interval, QRS-duration, QT-interval, QTcF-interval, and the interpretation of the ECG profile by the Investigator.

3.5.1.2.5 Physical Examination

Physical examination will be performed according to the clinical site's SOPs. In addition, body weight and height will be measured according to the clinical site's SOPs. A complete physical examination will be conducted at screening. After screening, physical examinations will be targeted, focusing on specific organ systems, abnormalities identified on the screening examination, and abnormalities related to AEs. Targeted physical examinations will be conducted at the other time points indicated in the schedule of assessments (Table 1). Symptom-directed physical examinations may also be conducted at any time, per the Investigator's discretion.

CCI [redacted]

CCI [redacted]

3.5.1.4 Total Blood Volume

Table 4 presents the number and volume of blood samples and the total volume of blood that is planned to be collected throughout the study per participant.

If deemed necessary by the Investigator or the Sponsor, the number and/or volume of blood samples per assessment may be increased, as long as the total volume of blood drawn for a participant does not surpass 600 mL (except when extra blood samples need to be taken for safety reasons).

Table 4 Number and Volume of Blood Samples and Total Blood Volume Planned to be Collected per Participant

Assessment	Maximum # Samples	Volume of Blood per Sample (mL)	Total Volume of Blood (mL)
PK of GBT021601 in:			
- plasma	30	3	90
- whole blood	30	1	30
PK of total radioactivity in:			
- plasma (predose)	1	10	10
- plasma (postdose)	29	2	58
- whole blood (predose)	1	4	4
- whole blood (postdose)	29	1	29
CCI [redacted]	[redacted]	[redacted]	[redacted]
Metabolite identification (including backup samples) in:			
- plasma	~~15~~ *22*	8	~~120~~ *176*
- whole blood	~~15~~ *22*	4	~~60~~ *88*
CCI [redacted]	[redacted]	[redacted]	[redacted]
Clinical chemistry	6	3.5	21
Hematology	6	3	18
Coagulation	1	2.7	2.7
Serology	1	5	5
Total volume of blood drawn			~~**450.7**~~ ***534.7***

PK=pharmacokinetics; CCI [redacted]

Note: the blood volumes may be adapted before the start of the study (eg, based on method development or availability of tubes), without exceeding the total amount of 600 mL blood per participant.

3.5.2 Appropriateness of Measurements

The assessments that will be made in this study are standard and generally recognized as reliable, accurate, and relevant.

3.5.2.1 Timing of Assessments

For PK, the predose samples will be obtained within 60 minutes before dosing. Postdose samples up to 20 minutes postdose will be obtained with a time window of ±1 minute. Thereafter, postdose samples will be obtained with time margins of ±5% of the time that has passed since dosing. The ±5% time window also applies to the start and end times of urine collection intervals and in addition to the total duration of each collection interval.

For safety assessments, predose assessments will be performed between waking up and dosing. For safety assessments up to 2.5 hours postdose, a time window of ±15 minutes is allowed. Thereafter, serial postdose assessments (eg, multiple assessments within any given day) will be performed with time margins of ±10% of the time that has passed since dosing.

When assessments are planned for the same scheme time, the order of the assessments should be arranged in such a way that PK blood sampling will be done after the ECG and vital signs recordings have been conducted, with PK blood sampling exactly at the scheduled time.

3.5.3 Pharmacokinetic and Safety Variables

3.5.3.1 Pharmacokinetic Variables

Pharmacokinetic variables will be the whole blood, plasma, and urine concentrations of GBT021601, and whole blood, CCI plasma, urine, and fecal (and vomitus, if applicable) concentrations of [^{14}C]-GBT021601 total radioactivity, and corresponding PK parameters. CCI [redacted] The PK parameters to be determined or calculated using noncompartmental analysis include, but are not limited to, the parameters as given in Table 5. Additional details about the PK analysis and a complete list of PK parameters will be provided in the statistical analysis plan (SAP).

Metabolite identification endpoints, including the structural identification of selected metabolites derived from [^{14}C]-GBT021601 in whole blood and plasma samples, and selected, pooled urine and fecal samples, will be reported separately.

Table 5 Pharmacokinetic Parameters

[^{14}C]-GBT021601 Total Radioactivity and GBT021601 in Whole Blood and Plasma	
Parameter	**Description**
C_{max}	Maximum observed plasma concentration
t_{max}	Time to attain maximum observed plasma concentration
AUC_{0-24}	Area under the plasma concentration-time curve up to 24 hours postdose
AUC_{0-t}	Area under the plasma concentration-time curve up to time t, where t is the last point with concentrations above the lower limit of quantification
AUC_{0-inf}	Area under the plasma concentration-time curve from time 0 to infinity calculated as: $AUC_{0-inf}=AUC_{0-t}+C_{last}/k_{el}$, where C_{last} is the last measurable plasma concentration
k_{el}	Apparent terminal elimination rate constant (λ_z)
$t_{1/2}$	Apparent terminal elimination half-life, calculated as $0.693/k_{el}$
CL/F	Apparent total clearance, calculated as dose/AUC_{0-inf} (GBT021601 in whole blood and plasma only)
V_z/F	Apparent volume of distribution at terminal phase (GBT021601 in whole blood and plasma only)
AUC B:P	Blood to plasma ratio for total radioactivity and GBT021601 based on AUC
C_{max} B:P	Blood to plasma ratio for total radioactivity and GBT021601 based on C_{max}
CCI [redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	
[redacted]	[redacted]
[^{14}C]-GBT021601 Total Radioactivity and GBT021601 in Urine	
Parameter	**Description**
CL_R	Renal clearance (GBT021601 only)
Ae_{urine}	Cumulative amount of drug excreted in urine at each interval
fe_{urine}	Fraction of the dose administered excreted in urine (%) at each interval
[^{14}C]-GBT021601 Total Radioactivity in Feces (and Vomitus, if Applicable)	
Parameter	**Description**
Ae_{feces}	Cumulative amount of drug excreted in feces at each interval
Ae_{vomit}	Cumulative amount of drug excreted in vomitus (if applicable)
fe_{feces}	Fraction of the dose administered excreted in feces (%) at each interval
fe_{vomit}	Fraction of the dose administered excreted in vomitus (%), if produced
Ae_{total}	Total amount of drug excreted in urine and feces
fe_{total}	Total fraction of the dose administered excreted in urine and feces

B=blood; P=plasma CCI [redacted]

3.5.3.2 Safety Variables

The safety variables to be measured after dosing include, but are not limited to, the variables as given below. A complete list of safety variables will be provided in the SAP.

- Adverse events
- Clinical laboratory (clinical chemistry, hematology, and urinalysis)
- Vital signs (supine blood pressure and pulse, respiratory rate, and body temperature)
- 12-lead ECGs

3.5.4 Drug Concentration Measurements

Total Radioactivity

The analysis of total radioactivity in whole blood, plasma, urine, and fecal (and vomitus, if produced) samples will be performed by TNO (Zeist, The Netherlands) under responsibility of ICON, using a validated AMS assay. The bioanalytical report(s) for these determinations will be included in the clinical study report (CSR).

The AMS isotope ratio will be converted to ng-eq/mL of whole blood, plasma, urine, or fecal homogenate (and vomitus, if applicable). The total radioactivity concentrations in urine and feces will be used to determine the percentage of dose recovered in urine and feces based upon the radioactive dose administered to each participant.

CCI [redacted]

GBT021601

The analysis of GBT021601 in whole blood, plasma, and urine samples will be performed at PPD Bioanalytical Laboratories (Middleton, US) under responsibility of the Sponsor, using a validated liquid chromatography with tandem mass spectrometry assay. The bioanalytical report(s) for these determinations will be included in the CSR.

CCI [redacted]

Metabolite Profiling and Identification

Metabolite identification including the structural identification of selected metabolites derived from [^{14}C]-GBT021601 in whole blood and plasma samples, and selected, pooled urine and fecal samples will be performed by TNO (Zeist, The Netherlands) under responsibility of ICON, and in collaboration with the Sponsor. Pooling of biological samples will be conducted after initial results have been provided to Sponsor. Results will be reported separately from the CSR.

CCI [redacted]

3.5.5 Retention of Blood and Urine Samples

Clinical Laboratory Samples

Blood and urine samples remaining after clinical laboratory assessments have been performed can be used by ICON to develop and test methods. ICON will process these samples anonymously. These leftover samples will be stored for a maximum of 1 year after which the samples will be destroyed.

Pharmacokinetic Samples

After PK assessments have been performed, any remaining samples of whole blood, plasma, urine, and feces (and vomitus, as applicable) will be stored at the laboratories described in Section 3.5.4 for research related to this clinical study, future research into further development of GBT021601 and/or treatment methods, or future analyses of GBT021601, drug metabolism, and/or PK analyses. The samples will be stored for a maximum period of 3 years, after which the samples will be destroyed.

CCI [redacted]

3.6 Statistical Procedures and Determination of Sample Size

3.6.1 Analysis Sets

3.6.1.1 Safety Set

All participants who have received 1 dose of GBT021601.

3.6.1.2 Pharmacokinetic Set

All participants who have received 1 dose of GBT021601 and provided at least 1 measurable postdose PK concentration of GBT021601 or total radioactivity in whole blood or plasma.

3.6.2 Statistical and Analytical Plan for Pharmacokinetic and Safety Evaluation

A SAP will be generated by the Biostatistics Department of ICON; the SAP will be finalized prior to database lock. Full details of the analyses to be performed will be included in the SAP.

Any deviation from the SAP will be reported in the section "Changes in Planned Analysis" in the CSR.

3.6.2.1 Pharmacokinetic Evaluation

The PK parameters and their statistical evaluation will be included in the CSR for this study. All concentration data will be summarized using descriptive statistics and will be listed and summarized in tabular and/or graphical form. Missing PK data will not be imputed and any anomalous concentration values may be excluded from analysis at the discretion of the pharmacokineticist after review of available documentation. Any such exclusion will be discussed with the Sponsor's clinical pharmacologist and clearly described in the CSR. Additional details about data analysis will be provided in the SAP.

Individual whole blood and plasma PK parameters will be determined for both total radioactivity and GBT021601. Individual CCI and urine PK parameters will be determined for GBT021601. Individual urinary and fecal excretion of total radioactivity over time will be determined. The ratio of whole blood to plasma for total radioactivity and GBT021601, and the ratio of CCI to plasma for GBT021601 will also be calculated. CCI and ratio with total radioactivity in whole blood/plasma will also be determined.

Summary statistics of PK parameters (primary and secondary) will be presented including number of participants, arithmetic means, geometric means, standard deviations, coefficients of variation, medians, and ranges (minimum, maximum). No inferential statistical analyses are planned.

3.6.2.2 Evaluation of Safety and Tolerability

Demographics (age, sex, ethnicity, and race) and baseline characteristics (height, weight, and BMI) will be summarized. Medical history and previous concomitant medications will be provided in a data listing.

Safety and tolerability will be assessed through AEs, clinical laboratory, vital signs, ECGs, and physical examination findings, and any other parameter that is relevant for safety assessment. All safety data will be presented in listings, including any clinically significant abnormalities. Descriptive summaries will be provided for AEs, clinical laboratory tests, vital signs, and ECG data. Urinalysis results, concomitant medications, and physical examinations will be presented in listings only.

3.6.3 Determination of Sample Size

The sample size is not based on statistical considerations, but on a conventional number of participants that is considered sufficient to achieve the study objectives while accounting for potential dropout participants given the length of the study.

3.7 Data Quality Assurance

The study may be audited by the Quality Assurance Department at ICON to assess adherence to the clinical study protocol (CSP) and Quality System. During the conduct of the study, process-related audits may be performed. An audit certificate outlining any audits and other related activities performed may be provided in the appendices of the final CSR.

The clinical research site will be monitored by the study monitor to ensure correct performance of the study procedures and assure that the study is conducted according to the relevant regulatory requirements. The eCRF entries will be verified with the source documentation.

Regulatory authorities, the IEC, and/or the Sponsor's clinical quality assurance group may request access to all source documents, eCRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

Quality control principles will be applied throughout the performance of this study. Review procedures will be followed at ICON for all documents that are generated in relation with the study.

An explanation will be given for all missing, unused, and spurious data in the relevant sections of the CSR. Details on the handling of spurious and missing data will be included in the SAP.

4. ETHICS

4.1 Independent Ethics Committee

The submission package including the CSP and the ICFs will be submitted for review and approval by the IEC of the foundation "Beoordeling Ethiek Biomedisch Onderzoek" (English translation: "Assessment of Ethics of Biomedical Research") (PPD) prior to the eligibility screening. The composition of the IEC is in accordance with the recommendations of the World Health Organization, the International Council for Harmonisation (ICH) E6(R2) Guideline for Good Clinical Practice (GCP) (European Medicines Agency [EMA]/Committee for Medicinal Products for Human Use [CHMP]/ICH/135/1995),[10] and the EU Clinical Trial Directive (Directive 2001/20/EC)[11] (see below). The submission package will also be shared with the CA in the Netherlands for a statement of no objection.

ICON will keep the IEC informed about the progress of the study. All changes in research activities and all unanticipated problems involving risks to human participants will be immediately reported to the IEC. In accordance with Section 10, Subsection 1, of the Dutch law on Medical Research in Human Participants (WMO, revised Dec 2015),[12] ICON will inform the participants and the IEC if anything occurs on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal, or if further recruitment of participants in the study has been put on hold for that reason, whichever occurs first. The study may be suspended pending further review by the IEC, except insofar as suspension would jeopardize the participants' health. ICON will take care that all participants are kept informed.

No changes will be made in the study without IEC approval, except when required to eliminate apparent immediate hazards to human subjects.

Notification of the end of the study will be sent by ICON to the CA in The Netherlands and to the IEC within 90 days after completion of follow-up for the last participant. In case a study is temporarily halted, ICON will notify the IEC immediately, including the reason for this. In case a study is ended prematurely, ICON will notify the IEC and the CA in The Netherlands within 15 days, including the reasons for the premature termination. A summary of the results of the study will be sent by ICON to the CA and the IEC within 1 year after the end of the study.

4.2 Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin in the World Medical Association (WMA) Declaration of Helsinki, adopted by the 18th WMA General Assembly, Helsinki, Finland, Jun 1964, and subsequent amendments.[13]

This study is also designed to comply with ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995),[10] and the EU Clinical Trial Directive Directive 2001/20/EC[11], as incorporated into Dutch Law.[12]

Guidelines adopted by the ICH and other relevant international guidelines, recommendations, and requirements will be taken into account as comprehensively as possible, as long as they do not violate local law.

Whenever the term "Investigator" is noted in the CSP text, it may refer to either the Investigator at the site or an appropriately qualified, trained, and delegated individual of the investigational site.

4.3 Participant Information and Consent

All participants will be informed verbally and in writing regarding the objectives, procedures, and risks of study participation. The participants will have to sign the Dutch or English version of the ICF before any study-related procedures are started. The ICF contains information about the objectives of the study, the procedures followed during the study, and the risks and restrictions of the study, with special reference to possible side effects of the study drug and potential interactions. In addition, insurance coverage provided during the study is explained. The elements addressed in the ICF are according to the ICH E6(R2) Guideline for GCP (EMA/CHMP/ICH/135/1995).[10]

4.4 Privacy

All personal details will be treated as confidential by the Investigator and staff at ICON, the Sponsor, and any subcontractors involved, and handling of personal data will be in compliance with the EU General Data Protection Regulation.[14]

5. STUDY ADMINISTRATIVE STRUCTURE

5.1 Distribution of Activities

5.1.1 Preparation of Study Drug

The study drug will be prepared at the ICON Pharmacy (Groningen, The Netherlands).

5.1.2 Laboratory Assessments

The analysis of clinical laboratory samples will be performed at the ICON Clinical Laboratory (Groningen, The Netherlands).

The analysis of total radioactivity in whole blood, plasma, urine, and fecal (and vomitus, if produced) samples will be performed by TNO (Zeist, The Netherlands) as described in Section 3.5.4.

The analysis of GBT021601 in whole blood, plasma, and urine samples will be performed at PPD Bioanalytical Laboratories (Middleton, US) as described in Section 3.5.4.

Metabolite profiling and identification of [^{14}C]-GBT021601 in whole blood and plasma samples, and selected, pooled urine and fecal samples will be performed by TNO (Zeist, The Netherlands) in collaboration with the Sponsor, as described in Section 3.5.4.

CCI [redacted]

5.1.3 Electronic Case Report Design

The eCRF design will be performed with the computer program Medidata Rave® Electronic Data Capture (Medidata Solutions, New York, NY, US) by the Database Programming Department of ICON.

5.1.4 Data Management

Data management will be performed with the computer programs Medidata Rave® Electronic Data Capture (Medidata Solutions, New York, NY, US), SAS® (SAS Institute Inc, Cary, NC, US), and EXACT (Kinship EXACT™, Kinship Technologies, a technology subsidiary of ICON) by the Data Management Department of ICON.

5.1.5 Statistics

An SAP will be provided by the Biostatistics Department of ICON. The safety analysis and the statistical evaluation of PK parameters will be conducted by the Biostatistics Department of ICON. Statistical analysis will be performed with the computer program SAS® (SAS Institute Inc, Cary, NC, US). PK parameters will be calculated using Phoenix WinNonlin (Certara, Princeton, NJ, US). Additional PK computations can also be performed in SAS®.

5.1.6 Clinical Study Report Writing

The CSR, structured in accordance with the guideline "Structure and Content of Clinical Study Reports - ICH E3",[15] will be written by ICON.

5.2 Documentation

5.2.1 Archiving

All documents concerning the study will be kept on file in the Central Archives of ICON for at least 25 years after conduct of the study. The Sponsor will receive the completed eCRFs (upon request, as PDF file).

5.2.2 Recording of Data in Source Documents and Electronic Case Report Forms

A data management plan will be written by the Data Management Department of ICON, which will be finalized prior to performing any data validation. The generated study data at the clinical research center will be collected on paper source documents. The clinical data manager will provide data entry access to the designated staff for data entry into the eCRFs. The clinical data manager will provide study-specific eCRF completion guidelines to the clinic personnel to facilitate data entry and to the clinical research associates to facilitate source data verification. Printouts of laboratory reports and ECG tracings are appendices to the source documents and are kept in the participant charts in addition to any other required ancillary source documents. Screen failures will not be entered into the database for this study.

6. CONFIDENTIALITY AND PUBLICATION POLICY

All information generated in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from the Sponsor. However, authorized regulatory officials, the Sponsor and its authorized representatives are allowed full access to the records.

All study participants must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant, who will be required to give consent for their data to be used as described in the ICF. The participants must be informed that their medical records may be examined by auditors or other authorized personnel appointed by the Sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.

Identification of participants and eCRFs shall be by unique participant numbers only.

All personal details will be treated as confidential by the Investigator and staff at ICON.

All relevant aspects regarding publication will be part of the contract (or similar document) between the Sponsor and ICON.

7. REFERENCES

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4. Oxbryta (voxelotor) tablets and tablets for oral suspension prescribing information. South San Francisco, Calif. Global Blood Therapeutics, Inc.; Dec 2021.
5. Metcalf B, Chuang C, Dufu K, et al. Discovery of GBT440, an Orally Bioavailable R-State Stabilizer of Sickle Cell Hemoglobin. ACS Med Chem Lett. 2017;8(3):321-326.
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7. Brown R, Redfern A, Lisbon E, Washington C, Agodoa I, and Smith-Whitley K. GBT021601, a next generation HbS polymerization inhibitor: results of safety, tolerability, pharmacokinetics and pharmacodynamics in adults living with sickle cell disease and healthy volunteers. Presented at: American Society of Hematology Annual Meeting & Exposition; 11-14 Dec 2021.
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9. Clinical Pharmacology Considerations for Human Radiolabeled Mass Balance Studies, Draft Guidance. U.S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Clinical Pharmacology, May 2022.
10. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E6(R2): Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice. Adopted by the European Medicines Agency (EMA), Committee for Human Medicinal Products, Document Reference EMA/CHMP/ICH/135/1995), 14 Jun 2017.
11. Directive 2001/20/EC of the European Parliament and of the Council of 04 Apr 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of Good Clinical Practice in the conduct of clinical trials on medicinal products for human use.
12. Medical Research Involving Human Subjects Act (WMO, Wet Medisch-Wetenschappelijk Onderzoek met Mensen), revision Dec 2015.
13. World Medical Association (WMA) Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (18th WMA General Assembly 1964), revised at 64th WMA General Assembly, Fortaleza, Brazil, Oct 2013.

14. The General Data Protection Regulation (GDPR). Regulation (EU) 2016/679 of the European Parliament and the Council of the European Union, 27 Apr 2016, applicable as of 25 May 2018.
15. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E3: Structure and Content of Clinical Study Reports. Note for Guidance on Structure and Content of Clinical Study Reports, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/137/95, Jul 1996.
16. International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline, E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. Note for Guidance on Clinical Safety Data Management, Adopted by the Committee for Human Medicinal Products, European Medicines Agency (EMA), Document Reference CPMP/ICH/377/95, Jun 1995.

8. APPENDICES

8.1 Drug Accountability

The study drug will be kept in the ICON Pharmacy or in a locked and secured storage facility accessible to the pharmacist and the pharmacy assistant only.

The responsible pharmacist will keep an inventory. This will include a description of the formulation and the quantity of study drug received for the study and a record of what is dispensed, to whom, and when. Details of accountability oversight are in the Monitoring Plan.

On termination of the study, the responsible pharmacist will conduct a final inventory of the study drug supply and will record the results of this inventory in the Drug Accountability Form. Unused study drug will be locally destroyed according to the clinical site's SOPs.

8.2 Adverse Events and Serious Adverse Events Evaluation and Reporting

8.2.1 Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. AE definitions will be followed as stated in the "Note for Guidance on Clinical Safety Data Management: Definitions and Standards for Expedited Reporting" (ICH topic E2A).[16]

All AEs reported by the participants or apparent from their physical appearance during the clinical study will be reported on the AE eCRF page.

Whenever possible, the severity of AEs will be graded using the most recent version of the CTCAE 5-point scale:

- **Mild (Grade 1)**: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Moderate (Grade 2)**: Minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- **Severe (Grade 3)**: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
- **Life-threatening (Grade 4)**: Life-threatening consequences; urgent intervention indicated.
- **Death (Grade 5)**: Death related to AE.

If an AE has multiple aspects, the aspect with the highest severity will be graded.

It is emphasized that the term "severe" is a measure of severity; thus, a severe AE is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

In addition, clinically significant changes in physical examination findings and abnormal objective test findings (eg, laboratory, x-ray, ECG) should also be recorded as AEs. Test findings and physical examination findings can result in AEs if they:

- Are associated with accompanying symptoms, and/or
- Require additional diagnostic testing or medical/surgical intervention, and/or
- Lead to a change in study dosing or discontinuation from the study; result in the addition of significant additional concomitant drug treatment or other therapy, and/or
- Lead to any of the outcomes included in the definition of an SAE, and/or
- Are considered to be an AE by the Investigator or Sponsor.

Reporting as an AE should not be triggered by:

- Merely repeating an abnormal test, or
- Any abnormal test result that is determined to be an error.

The relationship of any AE to the study drug will be assessed and graded as related or not related:

Related:

- The AE follows a reasonable temporal sequence to study drug administration, and cannot be reasonably explained by the participant's clinical state or other factors (eg, disease under study, concurrent diseases, or concomitant medications).
- The AE follows a reasonable temporal sequence to study drug administration, and is a known reaction to the drug under study or a related chemical group, or is predicted by known pharmacology.

Not Related:

- The AE does not follow a reasonable sequence from study drug administration, or can be reasonably explained by the participant's clinical state or other factors (eg, disease under study, concurrent diseases, and concomitant medications). If an SAE is not considered to be related to study drug, then an alternative explanation should be provided.

8.2.2 Serious Adverse Events

An SAE is any untoward medical occurrence that, on the basis of medical and scientific judgment:

- Results in death, or
- Is life-threatening (this refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe), or

- Requires inpatient hospitalization for a medical reason or prolongation of existing hospitalization (this refers to hospital admission required for treatment of the AE). (Note: this does not include confinement in, for example, a respite unit; a skilled nursing unit; rehabilitation facility; the clinical research center; or confinement due to planned or unplanned reason unrelated to study), or
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization.

SAEs will be collected from signing the ICF until the EoS/ET visit. SAEs that continue beyond the normal collection period (ie, are ongoing at the time a participant exits the study) will be followed until resolution or until stabilized with sequelae, or until reasonable attempts to determine resolution of the event are exhausted. SAEs that begin after the participant's participation in the study is complete, may be reported at any time.

The Investigator or clinical site personnel must notify the Sponsor or designee of all SAEs and any urgent safety matters, regardless of relationship to the investigational drug, within 24 hours of clinical site personnel becoming aware of the event. The Investigator will provide the initial notification by sending a completed "SAE Notification Form," which must include the Investigator's assessment of the relationship of the event to investigational drug and must be signed by the Investigator.

In addition, notification is sent by ICON to the IEC and the participant's General Practitioner.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the Sponsor or designee.

All SAE reports should be sent to the Sponsor or designee, as per the instructions provided to the site.

8.2.3 Suspected Unexpected Serious Adverse Reactions

An SAE that is also an unexpected adverse drug reaction is called a suspected unexpected serious adverse reaction (SUSAR). Unexpected adverse reactions are adverse reactions of which the nature or severity is not consistent with the applicable product information (*ie, the most recent version of the IB for GBT021601*~~eg, IB for an unapproved investigational medicinal product~~).

The Sponsor or its representative (eg, ICON if agreed to before start of the study) will promptly report (expedited reporting) the following SUSARs to the IEC:

- SUSARs that have arisen in the current clinical study that was assessed by the IEC.
- SUSARs that have arisen in other clinical studies of the same Sponsor and with the same medicinal product, and that could have consequences for the safety of the participants involved in the current clinical study that was assessed by the IEC.

The Sponsor or designee will promptly report (expedited reporting) all SUSARs to the CA and the Medicine Evaluation Board of the country where this study is conducted and to the CAs in other Member States, as applicable. The Sponsor or designee will inform regulatory authorities, IECs, and Investigators, as applicable, of any events (eg, change to the safety profile of GBT021601, major safety findings that may place study participants at risk) that may occur during the clinical study that do not fall within the definition of a SUSAR but may adversely affect the safety of study participants.

SUSARs that have already been reported to the EMA Eudravigilance database do not have to be reported again to the CA and the Medicine Evaluation Board because they have direct access to the Eudravigilance database.

Expedited reporting will occur no later than 15 calendar days after the Sponsor or its representative has first knowledge of the adverse reactions. For fatal or life-threatening cases, the term will be maximally 7 calendar days for a preliminary report with another 8 calendar days for completion of the report.

8.2.4 Follow-up of Adverse Events

Follow-up of AEs will continue until resolution, stabilization, or death. In case of ongoing AEs at database lock, the data obtained at database closure will be used in the statistical analysis. The follow-up of the AE will be documented in the source documents and will be described in the final CSR only if considered relevant by the Investigator.

8.3 Pregnancy

A female clinical study participant should not be dosed and must be instructed to inform the Investigator immediately if she becomes pregnant during the study. Pregnancies occurring up to 140 days after dosing or up to the last 24-hour visit (if collection of blood and excretion is continued after this 140-day period) must also be reported to the Investigator or designee. The Investigator will make arrangements for the participant to be counseled by a specialist, to discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the outcome of the pregnancy is known and the child will also be followed for 3 months after delivery.

The Investigator should report all pregnancies of female clinical study participants to the Sponsor within 24 hours of becoming aware of them.

If the Investigator becomes aware of a pregnancy occurring in the partner of a male participant participating in the study up to 140 days after dosing or up to the last 24-hour visit (if collection of blood and excretion is continued after this 140-day period) of the male participant, the pregnancy should be reported to the Sponsor or designee within 24 hours. The Investigator may make arrangements for the partner to be counseled by a specialist, to discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the partner should continue until the outcome of the pregnancy is known and the child will also be followed for 3 months after delivery.