Clinical Trial Protocol	
EudraCT no.	2022-003822-29
Investigational medicinal product	Compound 21 (C21)
Trial code	VP-C21-012
Protocol version and date	Final v2.0; 28FEB2023

A single-centre, open-label, fixed-sequence trial to evaluate the impact of C21 on the exposure of CYP1A2, CYP2C9, CYP3A4 and P-gp substrates in healthy volunteers

Phase	Phase 1, Human pharmacology
Indication	N/A
Test product	C21
CYP1A2 substrate	Caffeine
CYP2C9 substrate	Tolbutamide
CYP3A4 substrate	Midazolam
P-gp substrate	Nintedanib
Sponsor signatories	[redacted]
Principal Investigator	[redacted]
Clinical trial conduct and management	Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden

1 TRIAL SYNOPSIS

Trial title A single-centre, open-label, fixed-sequence trial to evaluate the impact of C21 on the exposure of CYP1A2, CYP2C9, CYP3A4 and P-gp substrates in healthy volunteers.	
Trial code VP-C21-012	**EudraCT no.** 2022-003822-29
Planned trial period Q1 to Q2 2023	**Phase of development** Phase 1, Human pharmacology
Principal Investigator [redacted]	
Trial design This is a single-centre, open-label, fixed-sequence trial to evaluate the influence of C21 on the exposure of CYP1A2, CYP2C9, CYP3A4 and P-gp substrates in healthy volunteers.	
Objectives Primary objective • To evaluate the impact of C21 on the pharmacokinetics (PK) of caffeine, tolbutamide, midazolam, nintedanib, and their main metabolites. Secondary objectives • To evaluate the PK of C21 and its main metabolite M1. • To evaluate the safety of C21. Exploratory objectives • To explore the impact of C21 on endogenous biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I (biomarker for OATP1B1 inhibition). • To evaluate the metabolite profile of C21 at steady state in plasma of healthy subjects. • To evaluate how genetic factors may influence the PK of C21. Exploratory endpoints related to the biomarker coproporphyrin I will be reported in the clinical trial report (CTR). Other exploratory endpoints will not be reported in the CTR but may be included in separate reports.	
Endpoints Primary endpoint • PK variables (C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{inf}) for caffeine, tolbutamide, midazolam, nintedanib, and their metabolites paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, 1-hydroxy-midazolam, and BIBF 1202. Secondary endpoints • PK variables (C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{tau}) for C21 and M1. • Frequency, seriousness and intensity of adverse events (AEs). • Clinically significant changes in vital signs, electrocardiogram (ECG) and clinical laboratory measurements (haematology, clinical chemistry, coagulation).	

Exploratory endpoints

- Plasma concentration profiles of selected endogenous biomarkers of transporter inhibition and/or induction.
- Metabolite in safety testing (MIST) in plasma at steady state.
- Genotyping of genes encoding enzymes and/or transporters of relevance for investigational medicinal product (IMP) metabolism and/or transport.

Number of subjects planned

Approximately, 30 subjects are planned to be screened to achieve 18 included subjects and at least 16 treated and fully evaluable subjects, *i.e.*, subjects that complete the trial up until at least the end of Day 19.

Diagnosis and eligibility criteria

Healthy male or female volunteers, 18 to 60 years (inclusive) with a body mass index (BMI) of 18.5 to 30.0 kg/m^2 and who are willing and able to give written informed consent are considered eligible for participation in the trial.

Only women of non-childbearing potential and male subjects will be eligible for participation. Male subjects with female partners of child-bearing potential must agree to use a highly effective method of contraception. Subjects with a history of or present medical condition that may interfere with the conduct of the trial or may put the subject at risk because of participation in the trial will be excluded.

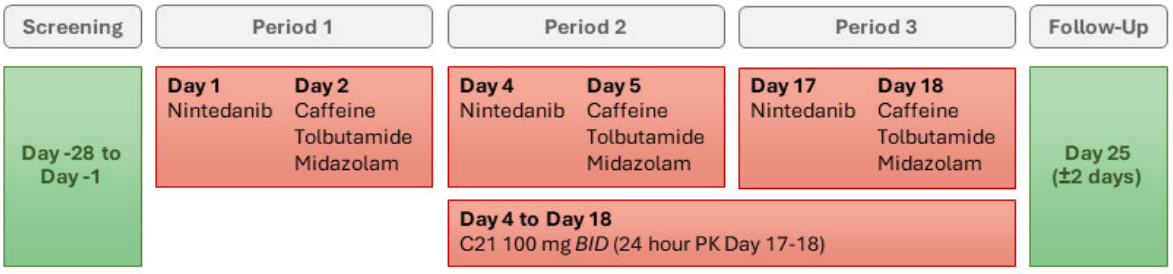
Methodology

The trial consists of a screening phase (Day -28 to Day -1), an open-label intervention phase (Day -1 to Day 19), and a follow-up phase (Day 20 to Day 25 [±2 days]). Subjects will remain at the trial site from the afternoon of Day -1 to the morning of Day 6, and again from the afternoon of Day 16 to the morning of Day 19.

The intervention phase consists of 3 periods: in period 1 (Day 1 to Day 3), the pharmacokinetics (PK) of all substrates will be evaluated in the absence of C21, in period 2 (Day 4 to Day 6), a potential inhibitory effect of C21 on the substrates be evaluated, and in period 3 (Day 17 to Day 19), the net effect of potential C21-mediated induction and inhibition on the substrates will be evaluated.

Subjects will be expected to attend a total of 4 visits to the trial site, including a screening visit (Visit 1), 2 intervention visits (Visits 2 and 3) and a follow-up visit (Visit 4).

An overview of the trial dosing schedule is shown below.



IMP, dosage and mode of administration

IMP	Type	Formulation Strength	Dose and no. of doses
C21	Test/perpetrator drug	Oral capsules 50 mg	100 mg *BID* Day 4 to Day 18.
Caffeine	CYP1A2 substrate	Oral tablets 100 mg	Single dose 100 mg on Days 2, 5 and 18.
Tolbutamide	CYP2C9 substrate	Oral tablets 500 mg	Single dose 500 mg on Days 2, 5 and 18.
Midazolam	CYP3A4 substrate	Oral solution 1 mg/mL	Single dose 2 mg on Days 2, 5 and 18.
Nintedanib	P-gp substrate	Oral soft capsules 150 mg	Single dose 150 mg on Days 1, 4 and 17.

Duration of IMP administration

The participating subjects will receive 3 single doses of nintedanib, on Days 1, 4, and 17 of the trial, as well as 3 single doses of the CYP substrate cocktail (caffeine, tolbutamide and midazolam) on Days 2, 5, and 18 of the trial. In total, participating subjects will receive 30 doses of C21, administered *BID*, over 15 days (Day 4 to Day 18).

Duration of each subject's involvement in the trial

Each subject is expected to participate in the trial for approximately 55 days, including an up to 28-day screening period, 19-day intervention period and a 4- to 8-day follow-up period.

Pharmacokinetic assessments

Blood samples for the determination of plasma concentrations and PK characterisation of caffeine, tolbutamide, midazolam, nintedanib and their metabolites (paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, 1-hydroxy-midazolam, and BIBF 1202), as well C21 and its main metabolite M1.

Safety assessments

AE reporting, vital signs (blood pressure and pulse rate), resting 12 lead ECG, blood sampling for the analysis of clinical chemistry, haematology and coagulation parameters, and physical examinations.

Exploratory assessments

Plasma concentration profiles of endogenous biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I.

MIST analysis of C21 in plasma at steady state.

Genotyping of genes encoding enzymes and/or transporters of relevance for C21 and substrate metabolism and/or transport (optional).

Statistical methods

No formal sample size calculation has been performed for this trial. The proposed sample size is considered sufficient to provide adequate information to meet the trial objectives.

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC). The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 8.3 or later (Certara, U.S.A.).

Baseline will be defined as the last non-missing data collection point prior to the first administration of IMP. All hypothesis testing will use a significance level of 5 %. No imputation of missing data will be performed.

PK parameters will be calculated by standard non-compartmental analysis (NCA). PK data will be presented by substance using summary statistics with number of measurements, arithmetic mean, SD, as well as median, minimum and maximum values. For AUC and C_{max} parameters, the geometric mean and geometric CV% will be presented. C_{max} and AUC ratios will be analysed using a paired t-test and summarised by substance using geometric means and their 90% confidence intervals.

Safety data will be summarised using descriptive statistics as appropriate.

Trial reporting

After completion of the trial, an International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E3 guideline-compliant CTR will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
ATC	Anatomical therapeutic chemical
ADL	Activities of daily living
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	Auxiliary medicinal product
ANC	Absolute neutrophil count
APTT	Activated partial thromboplastin clotting time
AR	Adverse reaction
AST	Aspartate aminotransferase
AT2R	Angiotensin II type 2 receptor
ATRAG	Angiotensin II type 2 receptor agonist
AUC	Area under the plasma concentration *vs.* time curve
$AUC_{0\text{-last}}$	AUC from 0 to time of last measurable plasma concentration
AUC_{inf}	AUC from 0 to infinity
AUC_{tau}	AUC from 0 to the end of the dosing interval
BCRP	Breast cancer resistance protein
B-EVF	Blood erythrocyte volume fraction (haematocrit)
BID	Twice daily (lat. *bis in die*)
BMI	Body mass index
CA	Competent authority
CIOMS	Council for International Organisations of Medical Sciences
C_{max}	Maximum observed concentration
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CTP	Clinical trial protocol
CTR	Clinical trial report
CV	Coefficient of variation
CYP	Cytochrome P450 enzymes
DDI	Drug-drug interaction

Abbreviation	Explanation
DMP	Data management plan
DSUR	Development safety update report
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EU	European Union
FDA	United States Food and Drug Administration
FIH	First-in-human
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
GCP	Good clinical practice
GDPR	General data protection regulation
GMP	Good manufacturing practice
Hb	Haemoglobin
HIV	Human immunodeficiency virus
HR	Heart rate
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
IMP	Investigational medicinal product
IPF	Idiopathic pulmonary fibrosis
ISF	Investigator site file
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MDR1	Multi-drug resistance protein 1
MedDRA	Medical dictionary for regulatory activities
MIST	Metabolites in safety testing
NCA	Non-compartmental analysis
NSAID	Non-steroidal anti-inflammatory drug

Abbreviation	Explanation
OAT3	Organic anion transporter 3
OATP1B1	Organic anion transporting polypeptide 1B.
PI	Principal Investigator
PII	Personally identifiable information
PK	Pharmacokinetics
PK(INR)	Prothrombin complex international normalised ratio
PKAS	PK analysis set
PKRAS	PK ratio analysis set
PPE	Personal protective equipment
PR interval	(ECG) The time from the onset of the P wave to the start of the QRS complex
PT	Preferred term
QA	Quality assurance
QC	Quality control
QP	Qualified person
QRS interval	(ECG) The time required for stimulus to spread through the heart's ventricles
QT interval	(ECG) The time from the beginning of the QRS complex to the end of the T wave
QTcF	(ECG) Corrected QT interval by Fredericia
QTcR	(ECG) Corrected QT interval by Rautaharju
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SD	Standard deviation
SDV	Source data verification
SLC	Solute carrier
SMP	Safety management plan
SmPC	Summary of product characteristics
SOC	System organ class
SOP	Standard operating procedures
SSc	Systemic sclerosis
SuHx	Sugen-Hypoxia
SUSAR	Suspected unexpected serious adverse reaction
T_{max}	Time of occurrence of C_{max}

Abbreviation	Explanation
TMF	Trial master file
WHO	World Health Organization
WHODrug	WHO Drug Dictionary

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contact

The Principal Investigator (PI) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the trial. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.5.1.14.

In the case of a medical emergency, the Investigator may, during office hours, contact the Medical Monitor (Table 1).

Table 1 Medical emergencies contact

Name	Function in the trial	Telephone number	E-mail
[redacted]	[redacted]	[redacted]	[redacted]

5 INVESTIGATOR AND TRIAL ADMINISTRATIVE STRUCTURE

Sponsor

Vicore Pharma AB
Kornhamnstorg 53
SE-111 27 Stockholm, Sweden

Sponsor's signatories

[redacted]

Sponsor's project manager

[redacted]

Sponsor's trial physician

[redacted]

Clinical conduct (trial site)

Clinical Trial Consultants AB

CTC Oscar,
Dag Hammarskjölds väg 10C
SE-752 37 Uppsala, Sweden

Principal Investigator

[redacted]

Trial management

Clinical Trial Consultants AB

Dag Hammarskjölds väg 10B
SE-752 37 Uppsala, Sweden

Clinical research manager

[redacted]

Biostatistician

[redacted]

	Pharmacokineticist [redacted]
	Medical Writer (Author of the clinical trial protocol [CTP]) [redacted]
	Medical Monitor [redacted]
Pharmacovigilance services	PrimeVigilance 1 Occam Court Surrey Research Park Guildford, Surrey GU2 7HJ
Laboratory (HIV and hepatitis)	Clinical Microbiology Uppsala University Hospital Dag Hammarskjölds väg 38 SE-752 37 Uppsala, Sweden
Laboratory (safety parameters, *CYP2C9* genotyping)	Clinical Chemistry and Pharmacology Uppsala University Hospital Entrance 61, 2nd level SE-751 85 Uppsala, Sweden
Laboratory (bioanalysis)	Lablytica Life Science AB Virdings Allé 16 SE-754 50 Uppsala, Sweden
Laboratory (MIST)	MetaSafe Sweden AB Biovation Park B215 Forskargatan 20J SE-151 36 Södertälje, Sweden
Manufacturing, packaging, labelling, and release of test product (C21)	Ardena Gent NV Kleimoer 4 B-9030 Mariakerke (Gent), Belgium
Import of CYP2C9 substrate (tolbutamide)	ClinStorage AB Banvaktsvägen 22 SE-171 48 Solna, Sweden
Trial pharmacy	Apoteket AB Nationella Enheten Uppsala Clinical Trial Unit Södra Depågatan 2 SE-754 54 Uppsala, Sweden

Electronic data capture (EDC) system provider	Viedoc Technologies AB Stationsgatan 23 SE-753 40 Uppsala, Sweden

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Vicore Pharma's Compound 21 (C21), 3-[4-(1H-imidazol-1-ylmethyl)phenyl]-5-(2-methylpropyl)thiophene-2-[(N-butyloxylcarbamate)-sulphonamide] sodium salt, is a potent and selective nonpeptide angiotensin II type 2 receptor (AT_2R) agonist, in clinical development for oral treatment of coronavirus disease 2019 (COVID-19) and rare lung diseases including idiopathic pulmonary fibrosis (IPF). C21 has been granted orphan drug designation in IPF by the European Medicines Agency (EMA) and the United Stated Food and Drug Administration (FDA).

6.1.1 Non-clinical data

C21 exerts antifibrotic effects in several *in vivo* and *in vitro* models of fibrosis. In both the bleomycin -and monocrotaline-induced rat models of pulmonary fibrosis, C21 attenuated pulmonary fibrosis and within the monocrotaline model, C21 also reversed it. In addition, C21 restored cardiac function and reduced pulmonary vascular remodelling and pulmonary hypertension. The antifibrotic effects were in several studies accompanied by anti-inflammatory as well as antiproliferative effects. In the Sugen-Hypoxia (SuHx) model of pulmonary hypertension, C21 caused a robust reduction of the SuHx-induced increase in endothelial cell hyperplasia and pulmonary hypertension.

[redacted]

[redacted]

More detailed information is found in the current version of the C21 investigator's brochure (IB).

6.1.2 Clinical experience

C21 is being developed for oral treatment of COVID-19 and rare lung diseases, including IPF. It represents a novel treatment capable of reducing pulmonary inflammation, proliferation, fibrosis, and vasculopathy.

The safety and pharmacokinetics of C21 have been investigated in 3 phase 1 trials in healthy or obese subjects (VP-C21-001-16, VP-C21-002-16, and C21-003), a mechanistic phase 1 trial investigating the effect of intra-arterial infusions on forearm blood flow (VP-C21-009) and 2 phase 2 trials in subjects with COVID-19 (VP-C21-006, the Angiotensin II Type Two Receptor Agonist COVID-19 Trial [ATTRACT]) and in subjects with Raynaud's phenomenon (RP) secondary to systemic sclerosis (SSc) (VP-C21-004). In total, 151 subjects have been exposed to at least 1 dose of C21 in the completed trials. Overall, C21 has been well-tolerated at doses up to 100 mg twice daily (*BID*) administered for up to 8 days.

The safety and efficacy of C21 in subjects with COVID-19 have been investigated in a multicentre, randomised, placebo-controlled, double-blind phase 2 trial (VP-C21-006). The trial included 106 adult subjects hospitalised with COVID-19, having a C-reactive protein (CRP) concentration of 50-150 mg/L, and not in need of mechanical ventilation. The subjects were randomised to receive oral treatment with C21 (100 mg twice daily, n=51) or placebo (n=55) for 7 days on top of SoC. C21 was well tolerated with a good safety profile and decreased the need for supplemental oxygen therapy compared with placebo in subjects with COVID-19. A phase 3 trial to confirm the efficacy and safety of C21 in hospitalised subjects with COVID-19 has been clinically completed and the final report is pending (VP-C21-008). The trial was a randomised, double blind, placebo-controlled, parallel-group, two-arm, multicentre trial investigating the efficacy and safety of C21 versus placebo as add-on therapy to local SoC in 272 subjects with COVID-19.

One phase 2 trial is currently ongoing. This is a multicentre, open-label, single-arm trial investigating the safety, tolerability, pharmacokinetics, and efficacy of C21 in subjects with IPF (VP-C21-005). Approximately 60 subjects are planned to be administered 100 mg C21 orally twice daily for a maximum of 36 weeks. [redacted]

[redacted]

6.2 Trial rationale

C21 is a first-in-class, low molecular weight, orally available, potent and selective angiotensin II type 2 receptor agonist (ATRAG) in clinical development for treatment of rare lung diseases, including IPF.

[redacted]

The purpose of this trial is to investigate the impact of C21 on the metabolic capabilities of CYP1A2, CYP2C9 and CYP3A4 using the following substrates administrated as a cocktail: caffeine (CYP1A2), tolbutamide (CYP2C9) and midazolam (CYP3A4). Furthermore, the influence of C21 on intestinal P-gp will be evaluated using nintedanib. Nintedanib is frequently used as standard of care in IPF, and thus has co-administration potential with C21. The selected substrates have been used in DDI trials previously and can be found in several publications (see Section 8.2).

The duration of C21 administration was selected based on the turnover of the enzymes to be investigated (*i.e.*, sufficient to capture a potential induction).

6.3 Risk/benefit assessment

6.3.1 General risk/benefit assessment

As the healthy volunteers in this trial will have no medical benefit from participation, their safety and wellbeing are of utmost importance.

[redacted] Overall, based on the available clinical experience, the risks associated with short-term administration of C21 100 mg *BID* in healthy volunteers are considered low.

C21 100 mg *BID* has been shown to exert beneficial effects in the lungs, as evidenced by the effect on the FVC in the ongoing phase 2 trial in IPF (VP-C21-005) and the effects on the requirement for oxygen supplementation in the completed phase 2 trial in COVID-19 (VP-C21-006).

The substrates used in the trial, *i.e.*, caffeine, tolbutamide, midazolam, and nintedanib are well known substances and the proposed single dose administration of these substrates is considered safe.

Subjects will remain at the trial site for at least 24 hours after co-administration of C21 and any of the substrates, and will be closely monitored by medical staff. Site visits may be prolonged in the event that the Investigator finds it medically warranted for safety reasons.

In cases of accidental overdose, subjects will be monitored appropriately, and standard supportive measures will be adopted as required. For further information regarding overdosing, refer to Section 11.5.1.18.

Each volunteer will be provided with a subject information card with information about the subject's participation in a trial, see Section 14.4.

The PI at the trial site will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the trial. The medical staff at the trial site have extensive experience from phase 1 and first-in-human (FIH) studies, and there are adequate procedures in place to handle unexpected and expected adverse reactions in the trial subjects.

Aside from the risks related to C21 and the substrates used in the trial, as detailed above, there may also be risks related to the medical devices used in the trial (*e.g.*, indwelling venous catheters). However, these devices are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. Trial-specific evaluations and sampling procedures, such as blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable. Overall, the combined safety data from previous pre-clinical and clinical studies have not revealed any safety issues that would outweigh the expected benefits of the trial.

While keeping the above-mentioned risk factors at a minimum level in order to not expose the subjects participating in the trial to risks that would not be ethically justifiable, it is concluded that the planned trial assessments are considered sufficient to meet the scientific and medical goals for the trial. It is therefore concluded that the potential benefits from the trial will outweigh the potential risks for the treated subjects.

More detailed information about the known and expected benefits and risks and reasonably expected adverse reactions (ARs) is found in the current version of the C21 IB. For detailed information, including safety information, on the CYP and P-gp substrate investigational medicinal products (IMPs) (caffeine, tolbutamide, midazolam, and nintedanib, see Table 10), refer to the corresponding summaries of product characteristics (SmPC) [3-6].

7 TRIAL OBJECTIVES AND ENDPOINTS

Table 2 Trial objectives and endpoints

Primary objective	**Endpoints**	**Assessments**	**Analysis**
To evaluate the impact of C21 on the pharmacokinetics (PK) of caffeine, tolbutamide, midazolam, nintedanib, and their main metabolites.	PK variables (C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{inf}) for caffeine, tolbutamide, midazolam, nintedanib, and their metabolites paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, 1-hydroxy-midazolam, and BIBF 1202.	Plasma sampling for PK analysis (Sections 11.3 and 11.4.1).	Section 17.5.1
Secondary objectives	**Endpoints**	**Assessments**	**Analysis**
To evaluate the PK of C21 and its main metabolite M1.	Pharmacokinetic variables (C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{tau}) for C21 and M1.	Plasma sampling for PK analysis (Section 11.3 and 11.4.1).	Section 17.5.1
To evaluate the safety of C21.	Frequency, seriousness and intensity of adverse events (AEs).	AE reporting (Section 11.5.1).	Section 17.6.1
	Clinically significant changes in vital signs, ECG and clinical laboratory measurements (haematology, clinical chemistry, coagulation).	Vital signs (Section 11.5.2).	Section 17.6.3
		Safety ECG (Section 11.5.3).	Section 17.6.4
		Clinical laboratory assessments (Section 11.5.4).	Section 17.6.5
Exploratory objective	**Endpoints**	**Assessments**	**Analysis**
To explore the impact of C21 on endogenous biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I (biomarker for OATP1B1 inhibition).	Plasma concentration profiles of selected endogenous biomarkers of transporter inhibition and/or induction.	Plasma sampling for biomarker analysis (Sections 11.3 and 11.6.1).	Section 17.7.
To evaluate the metabolite profile of C21 at steady state in plasma of healthy subjects.	Metabolite in safety testing (MIST) in plasma at steady state.	MIST (Section 11.6.2).	Section 17.7.
To evaluate how genetic factors may influence the PK of C21	Genotyping of genes encoding enzymes and/or transporters of relevance for C21 and substrate metabolism and/or transport.	Plasma sampling for analysis of genetic factors (Section 11.6.3).	Section 17.7.

IMP=investigational medicinal product, OATP1B1=organic anion transporting polypeptide 1B, PK=pharmacokinetic(s).

Exploratory endpoints related to the biomarker coproporphyrin I will be reported in the clinical trial report (CTR). Other exploratory endpoints will not be reported in the CTR but may be included in separate reports.

8 TRIAL DESIGN

8.1 Overall trial design and schedule of events

This is a single-centre, open-label, fixed-sequence trial to evaluate the influence of C21 on the exposure of CYP1A2, CYP2C9, CYP3A4 and P-gp substrates in healthy male and female volunteers.

The trial consists of a screening phase (Day -28 to Day -1), an open-label intervention phase (Day -1 to Day 19), and a follow-up phase (Day 20 to Day 25 [±2 days]). Subjects will remain at the trial site from the afternoon of Day -1 to the morning of Day 6, and again from the afternoon of Day 16 to the morning of Day 19.

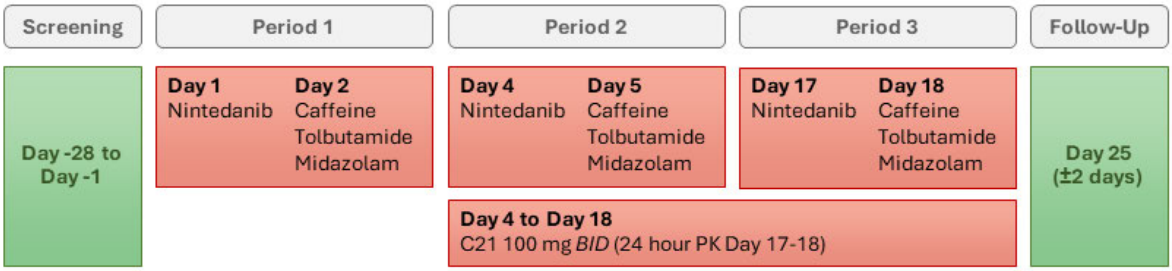
The intervention phase consists of 3 periods: in period 1 (Day -1 to Day 3), the pharmacokinetics (PK) of all substrates will be evaluated in the absence of C21, in period 2 (Day 4 to Day 6), a potential inhibitory effect of C21 on the substrates be evaluated, and in period 3 (Day 17 to Day 19), the net effect of potential C21-mediated induction and inhibition on the substrates will be evaluated (Table 3).

Subjects will be expected to attend a total of 4 visits to the trial site, including a screening visit (Visit 1), 2 intervention visits (Visits 2 and 3) and a follow-up visit (Visit 4).

Each subject is expected to participate in the trial for approximately 55 days, including an up to 28-day screening period, 19-day intervention period and a 4- to 8-day follow-up period.

The overall schedule of events is presented in Table 3 while an overview of the trial dosing schedule is shown in Figure 1. Trial assessments are described in Section 11.

Figure 1 Overview of the trial dosing schedule



8.1.1 Visit 1 – Screening

Screening (Visit 1) will take place from Day -28 to Day -1 and will include an eligibility check, a general health assessment (see Table 3 for details) and signing of the informed consent form (ICF). Eligible subjects will also be sampled to identify *CYP2C9* variant genotypes (refer to exclusion criterion no. 9). Adverse events (AEs) will be collected from the subject's signing of the ICF.

8.1.2 Visit 2 – First intervention visit

At Visit 2, the subjects will be admitted to the trial site in the afternoon/evening of Day -1 and will remain at the trial site until the morning of Day 5. Visit 2 encompasses period 1 and period 2.

On Day 1, after pre-dose assessments, the subjects will be administered a P-gp substrate (nintedanib 150 mg) in a fasted state, followed by blood sampling for PK and biomarker analyses, as well as safety assessments. The PK profile of nintedanib and its metabolites, *e.g.*, BIBF 1202, in the absence of C21 will be assessed until 48 hours post-dose. The plasma profile of biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I, will be assessed until 12 hours post-dose.

A detailed schedule of events of Day -1 to Day 1 is presented in Table 4.

On Day 2, after pre-dose assessments, the subjects will be administered a cocktail of CYP enzyme substrates (caffeine 100 mg, tolbutamide 500 mg and midazolam 2 mg) in a fasted state, followed by PK sampling and safety assessments. The PK profile of caffeine, tolbutamide, midazolam, and their metabolites (paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, and 1-hydroxy-midazolam) in the absence of C21 will be assessed until 24 hours post-dose.

A detailed schedule of events of Day 2 to Day 3 is presented in Table 5.

On Day 4, after pre-dose assessments, the subjects will be administered nintedanib (150 mg) together with the first scheduled dose of C21 100 mg (2x50 mg capsules) in a fasted state, followed by PK sampling and safety assessments. The PK profile of nintedanib and its metabolites in the presence of C21 will be assessed until 48 hours post-dose. The administration of C21 100 mg *BID* will continue for the subsequent 15 days (Day 4 to Day 18). A detailed schedule of events for Day 4 is presented in Table 6.

On Day 5, after pre-dose assessments, the subjects will be administered a cocktail of caffeine (100 mg), tolbutamide (500 mg), and midazolam (2 mg) together with the scheduled morning dose of C21 100 mg in a fasted state, followed by PK sampling and safety assessments. The PK profile of caffeine, tolbutamide, midazolam, and their metabolites in the presence of C21 will be assessed until 24 hours post-dose. On Day 6, the subjects will leave the trial site in the morning after PK sampling at 24 hours post-dose, the scheduled morning C21 dose and safety assessments.

A detailed schedule of events of Day 5 to Day 6 is presented in Table 7.

8.1.3 At-home self-administration of C21

From the evening of Day 6 until the morning of Day 16, subjects will self-administer C21 at home *BID* (morning and evening, a total of 20 doses) in a fasted state. Two remote check-ups for the collection of AEs and the use of concomitant medications, as well as verbal confirmation of C21 self-administration, will take place via telephone on Day 9 (±1 day) and Day 13 (±1 day).

8.1.4 Visit 3 – Second intervention visit

At Visit 3, the subjects will be admitted to the trial site in the afternoon/evening of Day 16 and will remain at the trial site until the morning of Day 19.

On Day 17, after pre-dose assessments, the subjects will be administered nintedanib (150 mg), together with the scheduled dose of C21 100 mg in a fasted state, followed by PK sampling and safety assessments. The PK profile of C21 and its main metabolite M1 at steady state will be assessed until 12-hours post-dose. The PK profile of nintedanib and its metabolites in the presence of C21 will be assessed until 48 hours post-dose. The plasma profile of biomarkers of transporter inhibition and/or induction will be assessed until 12 hours post-dose. Plasma samples from Day 17 will also be used for metabolites in safety testing (MIST).

A detailed schedule of events of Day 16 to Day 17 is presented in Table 8.

On Day 18, after pre-dose assessments, the subjects will be administered a cocktail of caffeine (100 mg), tolbutamide (500 mg) and midazolam (2 mg) together with the scheduled morning dose of C21 100 mg in a fasted state, followed by PK sampling and safety assessments. The PK profile of caffeine, tolbutamide, and midazolam in the presence of C21 will be assessed until 24 hours post-dose.

The last C21 dose will be given in the evening of Day 18. On Day 19, the subjects will leave the trial site in the morning after the 24-hour post-dose PK sampling and safety assessments.

A detailed schedule of events of Day 18 to Day 19 is presented in Table 9.

Table 3 Overall schedule of events

		Screening	Trial intervention period												Follow-up/End-of-trial[1]
			Period 1			Period 2				Telephone check-up[3]	Period 3				
Visit→	**CTP Section**	**Visit 1**	**Visit 2[2]**								**Visit 3[4]**				**Visit 4**
Assessment↓/Day→		**Day -28 to Day -1**	**Admission Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 9 + Day 13 ±1 day**	**Admission Day 16**	**Day 17**	**Day 18**	**Day 19**	**Day 25 ±2 days**
Informed consent	11.2.1	X													
Exploratory genetic sampling	11.6.3	X[5]													
Sampling for *CYP2C9* genotyping[6]	11.2.9	X													
Eligibility criteria	11.2.2	X	X[7]												
Demographics	11.2.3	X													
Weight/height (BMI)	11.2.4	X													X
Medical/surgical history	11.2.5	X													
HIV, hepatitis B and C	11.2.6	X													
Urine drug screen and alcohol test[8]	11.2.7	X	X[9]								X[9]				X
Physical examination[10]	11.2.8	X	X[9]						X		X[9]			X	X
Vital signs and safety ECG	11.5.2 11.5.3	X	X[9]						X		X[9]			X	X
Clinical laboratory profile	11.5.4	X	X[9]						X		X[9]			X	X
IMP administration (C21)	10.6						---------- X ----------								
C21 compliance check	10.8									X[3]	X[9]				
IMP administration (nintedanib)	10.6			X			X					X			
IMP administration (caffeine, tolbutamide, midazolam cocktail)	10.6				X			X					X		
Blood sampling for PK, biomarker analysis and MIST[11]:	11.3			X	X	X	X	X	X			X	X	X	
PK samples	11.4.1			x	x	x	x	x	x			x	x	x	
Biomarker samples	11.6.1			x								x			
MIST samples	11.6.2			x								x[12]			
Standardised meals[13]	9.6.1		X	X	X	X	X	X	X			X	X	X	

Visit→	CTP Section	Screening	Trial intervention period											Follow-up/End-of-trial[1]
			Period 1			Period 2				Telephone check-up[3]	Period 3			
		Visit 1	Visit 2[2]								Visit 3[4]			Visit 4
Assessment↓/Day→		Day -28 to Day -1	Admission Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 9 + Day 13 ±1 day	Admission Day 16	Day 17	Day 18	Day 19	Day 25 ±2 days
Overnight stays at the trial site			---------- X ----------								---------- X ----------				
Adverse events[14]	11.5.1	---------- X ----------													
Prior and concomitant medications	11.2.10	---------- X ----------													

AE=adverse event, BMI=body mass index, CTP=clinical trial protocol, ECG=electrocardiogram, HIV=human immunodeficiency virus, IMP=investigational medicinal product, MIST=metabolites in safety testing.

1. Or after early withdrawal.
2. Detailed schedule of events for Visit 2 is shown in Table 5 (Day -1 to Day 1), Table 4 (Day 2 to Day 3), Table 6 (Day 4), and Table 7 (Day 5 to Day 6).
3. Telephone check-ups on Day 9 (±1 day) and Day 13 (±1 day) will include a verbal confirmation of C21 self-administration as well as the collection of any AEs and uses of concomitant medication.
4. Detailed schedule of events for Visit 3 is shown in Table 8 (Day 16 to Day 17), and Table 9 (Day 18 to Day 19).
5. Informed consent for exploratory genotyping may be obtained at any time after the subject has signed the main informed consent form. Sampling may be performed at any time during the trial.
6. Subjects with *CYP2C9* genotypes hetero- or homozygous for *CYP2C9*2* (Arg144Cys) and/or *CYP2C9*3* (Ile359Leu) variant alleles will be excluded from participation in the trial. Refer to exclusion criterion no. 9.
7. Confirmation of eligibility criteria. Can be done on Day -1 or Day 1 prior to IMP administration.
8. Drug and alcohol screens may also be performed at additional time-points, at the discretion of the Investigator.
9. Pre-dose assessments can be done on Day -1 or Day 1 (Visit 2), Day 16 or Day 17 (Visit 3), prior to IMP administration.
10. Complete physical examinations will be done at the screening visit (Visit 1) and upon end-of-trial (Visit 4). Symptom-driven physical examinations will be done upon discharge from the trial site on Day 6 and Day 19.
11. For detailed timing of blood sampling, including allowed time deviations, refer to Table 11 in Section 11.3.
12. MIST may be done on samples from other time points, not only Day 17, if considered appropriate. Reference sample will be collected at pre-dose on Day 1.
13. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administrations on Day 1, Day 2, Day 4, Day 5, Day 17 and Day 18, and until 4 hours post-dose. C21 should always be taken in a fasted state, *i.e.*, no food intake for at least 2 hours before and 1 hour after C21 intake. The latter applies to evening administrations of C21 in-clinic and to all at-home administrations.
14. AEs will be collected from the subject's signing of the ICF during Visit 1.

Table 4 Detailed schedule of events for Visit 2, Day -1 to Day 1

Day→	**Day -1**	**Day 1**													
Assessment↓/Time→	**Admission**	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**
Eligibility criteria	X[1]														
Physical examination	X[2]														
Urine drug screen and alcohol test	X[2]														
Clinical laboratory profile	X[2]														
Vital signs	X[2]														
12-lead ECG	X[2]														
IMP administration (nintedanib)			X												
Blood sampling:		X		X	X	X	X	X	X	X	X	X	X	X	X
Nintedanib[3] PK samples		x		x	x	x	x	x	x	x	x	x	x	x	x
Biomarker[4] samples		x		x	x	x	x	x	x	x	x	x	x	x	
MIST sample		x[5]													
Standardised meals[6]	X										---------- X ----------				
Adverse events[7]	---------- X ----------														
Prior and concomitant medications	---------- X ----------														

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic(s).

1. Confirmation of eligibility criteria. Can be done on Day -1 or Day 1 prior to IMP administration.
2. Can be done on Day -1 or Day 1 prior to IMP administration.
3. Including its metabolites, *e.g.*, BIBF 1202.
4. Biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I.
5. Reference aliquot for MIST (see Table 8).
6. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administrations (00:00), and until 4 hours post-dose. Meals Day -1: dinner and evening snack. Meals Day 1: lunch, snack, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively.
7. Any AEs with start date on the day of first IMP administration (Day 1) must be recorded with start time.

Table 5 Detailed schedule of events for Visit 2, Day 2 to Day 3

Day→	**Day 2**														**Day 3**
Assessment↓/Time→	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**	**24:00**
IMP administration (caffeine, tolbutamide, midazolam cocktail)		X													
Blood sampling:	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Nintedanib[1] PK samples	x[2]												x[2]		x[2]
Caffeine, tolbutamide, midazolam[3] PK samples	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Standardised meals[4]										---------- X ----------					X
Adverse events	---------- X ----------														
Concomitant medications	---------- X ----------														

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic(s).

1. Including its metabolites, *e.g.*, BIBF 1202.
2. Correspond to the 24-hour, 36-hour, and 48-hour post-dose time points for the nintedanib dose administered on Day 1 (see Table 4).
3. Including their metabolites paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, and 1-hydroxy-midazolam.
4. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administration, and until 4 hours post-dose. Meals Day 2: lunch, snack, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively. Meals Day 3: breakfast, lunch, snack, dinner and evening snack.

Table 6 Detailed schedule of events for Visit 2, Day 4

Day→	**Day 4**													
Assessment↓/Time→	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**
IMP administration (C21)		X											X	
IMP administration (nintedanib)		X												
Blood sampling:	X		X	X	X	X	X	X	X	X	X	X	X[1]	X
Nintedanib[2] PK samples	x		x	x	x	x	x	x	x	x	x	x	x	x
Standardised meals[3]										---------- X ----------				
Adverse events	---------- X ----------													
Concomitant medications	---------- X ----------													

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic(s).

1. 12:00 blood sample must be collected prior to evening administration of C21 (second *BID* dose).
2. Including its metabolites, *e.g.*, BIBF 1202.
3. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administration, and until 4 hours post-dose. Meals Day 4: lunch, snack, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively.

Table 7 Detailed schedule of events for Visit 2, Day 5 to Day 6

Day→	**Day 5**														**Day 6**
Assessment↓/Time→	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**	**24:00**
Physical examination[1]															X
Clinical laboratory profile															X
Vital signs															X
12-lead ECG															X
IMP administration (C21)		X											X		X
IMP administration (caffeine, tolbutamide, midazolam cocktail)		X													
Blood sampling:	X		X	X	X	X	X	X	X	X	X	X	X[2]	X	X
Nintedanib[3] PK samples	x[4]												x[4]		x[4]
Caffeine, tolbutamide, midazolam[5] PK samples	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Standardised meals[6]										---------- X ----------					X
Adverse events	---------- X ----------														
Concomitant medications	---------- X ----------														

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic(s).

1. Symptom-driven physical examination only.
2. 12:00 blood sample must be collected prior to evening administration of C21 (second *BID* dose).
3. Including its metabolites, *e.g.*, BIBF 1202.
4. Correspond to the 24-hour, 36-hour, and 48-hour post-dose time points for the nintedanib dose administered on Day 4 (see Table 6).
5. Including their metabolites paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide and 1-hydroxy-midazolam.
6. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administration, and until 4 hours post-dose. Meals Day 5: lunch, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively. Meals Day 6: breakfast.

Table 8 Detailed schedule of events for Visit 3, Day 16 to Day 17

Day→	**Day 16**	**Day 17**												
Assessment↓/Time→	**Admission**	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**
Physical examination	X[1]														
Urine drug screen	X[1]														
Alcohol test	X[1]														
Clinical laboratory profile	X[1]														
Vital signs	X[1]														
12-lead ECG	X[1]														
IMP administration (C21)	X		X											X	
IMP administration (nintedanib)			X												
Blood sampling:		X		X	X	X	X	X	X	X	X	X	X	X[2]	X
C21/M1 PK samples		x		x	x	x	x	x	x	x	x	x	x	x	
Nintedanib[3] PK samples		x		x	x	x	x	x	x	x	x	x	x	x	x
Biomarker[4] samples		x		x	x	x	x	x	x	x	x	x	x	x	
MIST[5] samples		x		x	x	x	x	x	x	x	x	x	x	x	
Standardised meals[6]	X										---------- X ----------				
Adverse events	---------- X ----------														
Concomitant medications	---------- X ----------														

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic(s).

1. Can be done on Day 16 or Day 17 prior to IMP administration.
2. 12:00 blood sample must be collected prior to evening administration of C21 (second *BID* dose).
3. Including its metabolites, *e.g.*, BIBF 1202.
4. Biomarkers of transporter inhibition and/or induction, *e.g.*, coproporphyrin I.
5. A reference aliquot of plasma will be collected at pre-dose on Day 1 (see Table 4). Additional MIST analysis may be done on samples from other time points, not only Day 17, if considered appropriate.
6. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administration, and until 4 hours post-dose. Meals Day 16: dinner and evening snack. Meals Day 17: lunch, snack, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively.

Table 9 Detailed schedule of events for Visit 3, Day 18 to Day 19

Day→	Day 18														Day 19
Assessment↓/Time→	**Pre-dose**	**00:00**	**00:15**	**00:30**	**00:45**	**01:00**	**01:30**	**02:00**	**03:00**	**04:00**	**06:00**	**08:00**	**12:00**	**16:00**	**24:00**
Physical examination[1]															X
Clinical laboratory profile															X
Vital signs															X
12-lead ECG															X
IMP administration (C21)		X											X		
IMP administration (caffeine, tolbutamide, midazolam cocktail)		X													
Blood sampling:	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Nintedanib[3] PK samples	x[4]												x[4]		x[4]
Caffeine, tolbutamide, midazolam[5] PK samples	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Standardised meals[6]										---------- X ----------					X
Adverse events	---------- X ----------														
Concomitant medications	---------- X ----------														

ECG: Electrocardiogram. PK: Pharmacokinetic(s).

1. Symptom-driven physical examination only.
2. 12:00 blood sample must be collected prior to evening administration of C21 (second *BID* dose).
3. Including its metabolites, *e.g.*, BIBF 1202.
4. Correspond to the 24-hour, 36-hour, and 48-hour post-dose time points for the nintedanib dose administered on Day 17 (see Table 8).
5. Including their metabolites paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide and 1-hydroxy-midazolam.
6. Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administration and until 4 hours post-dose. Meals Day 18: lunch, snack, dinner and evening snack. Lunch will be served at least 4 hours post-IMP administration. Snack, dinner and evening snack will be served approximately 6, 9 and 12 hours post-IMP administration, respectively. Meals Day 19: breakfast.

8.2 Rationale for trial design

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline M12 on drug interaction studies (EMA guideline EMEA/CHMP/ICH/652460/2022, draft 21 July 2022) [7] was considered when designing this trial.

The proposed dose of C21 for the present trial is 100 mg *BID*, which is the highest intended therapeutic dose. The C21 dose is based on results from multiple preclinical studies, clinical safety and efficacy data of C21 from completed clinical trials in healthy or obese subjects and in subjects with COVID-19, as well as preliminary safety and efficacy data from the ongoing phase 2 trial in subjects with IPF (VP-C21-005).

[redacted]

To start exploring the clinical DDI characteristics of C21 as a perpetrator, a DDI cocktail containing the index substrates caffeine (CYP1A2 substrate), tolbutamide (CYP2C9 substrate) and midazolam (CYP3A4 substrate) has been selected. The intended doses for the index substrates are 100 mg for caffeine, 500 mg for tolbutamide and 2 mg for midazolam. This cocktail of index substrates has previously been used in clinical DDI studies at similar or at higher doses [8, 9]. The PK profiles of caffeine, tolbutamide, midazolam, and their main metabolites, will be studied on Day 2 (baseline), Day 5 (potential inhibitory effects including time-dependent inhibition) and Day 18 (potential net effect of inhibition and induction). To maximize the potential DDI effects of C21 as perpetrator, a C21 dose of 100 mg *BID* for 15 consecutive days was selected. The duration of C21 administration (Day 4 to Day 18) was selected to allow for potential steady state conditions in respect to CYP induction.

In addition to the CYP index substrates, the P-gp substrate nintedanib will be included in the trial. Nintedanib is frequently used as standard of care in IPF, and therefore any impact of C21 on nintedanib is important to identify. Nintedanib was also selected based on previously demonstrated interactions with P-gp perpetrators and the co-administration potential with C21 for the treatment of IPF. Nintedanib is only metabolized by CYP enzymes to a minor extent [10, 11]. Thus, it is expected to be a useful index drug for the assessment of C21 influence of P-gp. Nintedanib exhibits a low DDI risk as a perpetrator [10, 11] but has not been evaluated together with caffeine, tolbutamide and midazolam in a cocktail approach. Consequently, a staggered dosing schedule of nintedanib will be used, it will be administered the day before the CYP substrate cocktail, *i.e.*, on Day 1, Day 4 and Day 17. The intended dose for nintedanib is 150 mg, which a commonly used therapeutic dose that previously has been evaluated in DDI studies with nintedanib as a victim drug [12, 13].

The exploratory biomarker for OATP1B activity coproporphyrin I has also been included in the trial to evaluate the influence of C21 on OATP1B transport activity. The lipid-lowering 3-hydroxy-3-metaglutaryl coenzyme A reductase inhibitors, or statins, are substrates of OATPB1B and are commonly used in patients with IPF, as cardiovascular co-morbidities are frequent. There is thus a co-administration potential with C21. Since it has been demonstrated that nintedanib neither inhibits OATP1B1 nor OATP1B3 [10] the interaction potential between nintedanib and coproporphyrin I is considered very low. Thus, it is considered appropriate to investigate the plasma profile of coproporphyrin I on the same days as nintedanib.

9 TRIAL POPULATION

Prospective approval of deviations from the eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

9.1 Recruitment

Subjects will be recruited from CTC's database of healthy volunteers, as well as from strategic marketing campaigns. Advertisements in social media and other media (newspapers, internet, radio, local distribution of flyers *etc.*) will be used to reach the target audience. The advertisement texts approved by the independent ethics committee (IEC) will be used to create all materials (digital, radio and/or print) for recruitment.

9.2 Screening and enrolment log

Investigators must keep a record of all screened subjects even if they were not subsequently included in the trial. This information is necessary to verify that subjects were selected without bias. The reason for screening failure should be stated for all subjects screened but not included and treated. The reason for withdrawal should be stated for all subjects that were included but did not complete the trial.

A screening number generated automatically in the electronic case report form (eCRF) will be allocated to each subject in connection to the informed consent process at the screening visit (Visit 1). The screening number will allow identification of subjects irrespective of their possible eligibility for the trial.

Eligible subjects will be assigned a 3-digit randomisation number (i.e., 101-118) prior to the first IMP administration.

If a subject cannot receive the planned dose of IMP within 28 days after screening (*i.e.*, the time interval between signing informed consent until first administration of IMP), the subject should be rescreened before proceeding in the trial.

9.3 Number of subjects

Approximately, 30 subjects are planned to be screened to achieve 18 included subjects and at least 16 treated and fully evaluable subjects, *i.e.*, subjects that complete the trial up until at least Day 19.

For the replacement of subjects who discontinue the trial, see Section 9.8.6.

9.4 Inclusion criteria

For inclusion in the trial, subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the trial.
2. Healthy male, or healthy female subject of non-childbearing potential, aged 18 to 60 years, inclusive.
3. Body mass index $\geq$ 18.5 and $\leq$ 30.0 kg/m^2 at the time of the screening visit.
4. Medically healthy subject without abnormal clinically significant medical history, physical findings, vital signs, ECG and laboratory values at the time of the screening visit, as judged by the Investigator.

5. Women of non-childbearing potential, *i.e.* pre-menopausal females who have undergone any of the following surgical procedures; hysterectomy, bilateral salpingectomy or bilateral oophorectomy, or who are post-menopausal defined as 12 months of amenorrhea (in questionable cases a blood sample with detection of follicle stimulating hormone [FSH] >25 IU/L is confirmatory).
6. Male subjects who are vasectomised, who are willing to use condoms or to practice sexual abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) to prevent pregnancy and drug exposure of a partner. Male subjects must also refrain from donating sperm from the first administration of IMP until 3 months after the last administration of IMP. Any female partner of a non-vasectomised male subject who is of child-bearing potential must use contraceptive methods with a failure rate of < 1% (see inclusion criterion no. 5) to prevent pregnancy from at least 2 weeks prior to the first administration of IMP to 4 weeks after the last administration of IMP.

9.5 Exclusion criteria

Subjects must not enter the trial if any of the following exclusion criteria are fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the trial, or influence the results or the subject's ability to participate in the trial.
2. Any clinically significant illness, medical/surgical procedure or trauma within 4 weeks of the first administration of IMP.
3. Malignancy within the past 5 years, with the exception of *in situ* removal of basal cell carcinoma.
4. Any planned major surgery within the duration of the trial.
5. Subjects who are pregnant, currently breastfeeding, or intend to become pregnant during the course of the trial.
6. Any positive result at the screening visit for serum hepatitis B surface antigen, hepatitis C antibodies and/or human immunodeficiency virus (HIV).
7. After 10 minutes supine rest at the screening visit, any vital signs values outside the following ranges:
   - Systolic blood pressure: <90 or >140 mmHg, or
   - Diastolic blood pressure <50 or >90 mmHg, or
   - Pulse <40 or >90 bpm
8. Prolonged QTcF (>450 ms), cardiac arrhythmias or any clinically significant abnormalities in the resting ECG at the screening visit, as judged by the Investigator.
9. *CYP2C9* genotype hetero- or homozygous for *CYP2C9*2* (Arg144Cys) and/or *CYP2C9*3* (Ile359Leu) variant alleles associated with altered CYP2C9 activity and tolbutamide metabolism [14], sampled at the screening visit.
10. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to any of the IMPs.

11. Regular use of any prescribed or non-prescribed medications, including antacids, analgesics, herbal remedies, *e.g.* St. John's wort, vitamins and minerals, within 2 weeks prior to the first administration of IMP, except occasional intake of paracetamol (maximum 2000 mg/day and not exceeding 3000 mg/week), as well as nasal decongestants without cortisone, antihistamine or anticholinergics for a maximum of 10 days, at the discretion of the Investigator.
12. Planned treatment or treatment with another investigational drug within 3 months prior to Day -1. Subjects consented and screened but not dosed in previous phase 1 studies are not to be excluded.
13. Regular current smokers or users of nicotine products. Irregular use of nicotine (*e.g.*, smoking, snuffing, chewing tobacco) less than 3 times/week is allowed before the screening visit.
14. Positive screening result for drugs of abuse or alcohol at the screening visit or on admission to the trial site prior to the first administration of the IMP.
15. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator.
16. Presence or history of drug abuse, as judged by the Investigator.
17. History of, or current use of anabolic steroids, as judged by the Investigator.
18. Excessive caffeine consumption defined by a daily intake of > 5 cups (1 cup = approximately 240 mL) of caffeine containing beverages, as judged by the Investigator.
19. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the last three months prior to screening.
20. The Investigator considers the subject unlikely to comply with trial procedures, restrictions and requirements.

9.6 Restrictions during the trial

Subjects must be willing to comply with the restrictions as outlined in 9.6.1 and 9.6.2.

9.6.1 General restrictions

1. Contraception requirements: See inclusion criterion #5.
2. Fasting: Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administrations on Day 1, Day 2, Day 4, Day 5, Day 17, and Day 18, and until 4 hours post-dose. C21 should always be taken in a fasted state, *i.e.,* no food intake for at least 2 hours before and 1 hour after C21 intake. The latter applies to evening administrations of C21 in-clinic and to all at-home administrations. Water will be allowed *ad libitum* at the trial site except from 1 hour pre-dose to 1 hour post-dose.

3. Meals and dietary restrictions: *At the trial site:* The IMP will be administered under fasting conditions and swallowed with approximately 240 mL of tap water on days detailed in the schedule of events (Table 3 to Table 9). Standardised meals will be served while the subjects are in the trial site. The meal selection is standardised in the sense that the nutritional content of the meals is similar at each timepoint of each treatment day at the trial site. Lunch is served approximately 4 hours post-dose. A mid-day snack, dinner, and an evening snack is served approximately 6 hours, 9 hours, and 12 hours after the morning IMP administration, respectively. Breakfast will be served on days where no IMP is administered. Water will be allowed *ad libitum* at the trial site except from 1 hour pre-dose to 1 hour post-dose.

   At home: C21 will be self-administered by the subject at home *BID* (morning and evening) from the evening of Day 6 until the morning of Day 16. The subjects will be instructed to avoid food/drinks from 2 hours prior to IMP intake until 1 hour after C21 intake.

4. Alcohol: The consumption of alcohol is not allowed from 48 hours prior to admission to the trial site for Visit 2 and until end-of-trial at Visit 4.
5. Drugs of abuse: The use of drugs of abuse is not allowed from the screening visit to the end-of-trial visit. In addition to the urine drug screens described in the schedule of events, additional random testing can be performed at the site visits.
6. Coffee, tea and other caffeine-containing beverages: No coffee, tea or other caffeine-containing beverages are allowed from 48 hours prior to admission to the trial site for Visits 2 and 3 or during these visits. Subjects may be served caffeine-free coffee or tea during Visit 2 and Visit 3.
7. Taurine-containing beverages: Beverages containing taurine, *e.g.,* energy drinks such as Red Bull and Monster Energy, are not allowed from the screening visit until the end-of-trial visit.
8. Nicotine: Smoking or the use of nicotine-containing products, including non-tobacco oral nicotine products, is not allowed from the screening visit until the end-of-trial visit.
9. Grapefruit and grapefruit-containing products: The consumption of grapefruit and/or grapefruit-containing products such as jams, jellies, preserves and fruit juices will not be allowed from the screening visit until the end-of-trial visit. This also includes Seville oranges, pomelo, exotic citrus fruits, and other grapefruit hybrids.
10. Exercise: The subjects must refrain from strenuous exercise, defined as greater than 70% of the maximal pulse rate for one hour or more, from screening visit to end-of-trial visit.
11. Blood donation: The subjects must not donate blood or plasma from the screening visit until 3 months after the final medical examination at the end-of-trial visit.
12. Participation in other clinical studies: The subjects are not allowed to participate in any other interventional clinical trial from the screening visit until the end-of-trial visit.

9.6.2 Prior and concomitant therapy

9.6.2.1 Prohibited medications

The regular use of any prescribed or non-prescribed medication within 2 weeks prior to the first administration of IMP until the end-of-trial visit is not allowed (except as detailed in Section 9.6.2.2), as judged by the Investigator. This includes, *e.g.*, anticoagulants (including but not limited to warfarin, heparin and derivative substances), non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen and naproxen, antacids, analgesics, herbal remedies, vitamins and minerals.

9.6.2.2 Allowed medications

- Paracetamol in doses up to 2000 mg/day for a maximum of 3 consecutive days. If this amount of paracetamol is insufficient for the treatment of the subject, withdrawal should be considered.
- Nasal decongestants without cortisone, antihistamine or anticholinergics for a maximum of 10 days.

Other medications considered necessary for the subject's safety and wellbeing may be given at the discretion of the Investigator. Following consultation with the Sponsor, the Investigator will determine whether or not the subject should continue in the trial.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical trial but do not fulfil all eligibility criteria and are not subsequently included in the trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated ICF and reason(s) for screening failure.

Subjects who do not meet the criteria for participation in this trial may be rescreened.

Re-screening can be performed if any of the following were reasons for screening failure or non-randomisation, as judged by the Investigator:

- Practical reasons.
- Non-significant medical conditions (*e.g.*, influenza, nasopharyngitis).
- Replacement subjects not used in a previous group of subjects.

For subjects who are re-screened, a new screening number will be assigned and new, signed ICF must be collected.

9.8 Subject withdrawal

9.8.1 General withdrawal criteria

Subjects are free to discontinue their participation in the trial at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent should be documented.

Subjects may be discontinued from the trial at any time at the discretion of the Investigator.

Reasons for discontinuation can include:

- Withdrawal of consent (subject decision).

- Severe non-compliance to trial protocol procedures, as judged by the Investigator and/or Sponsor.
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor.
- Withdrawal of informed consent to the use of biological samples as detailed in Section 12.5.

9.8.2 Electrocardiogram withdrawal criteria

IMP administration will be stopped for any subject meeting any of the ECG withdrawal criteria below, and the subject will be withdrawn from the trial. The same QT correction formula will be used to determine discontinuation throughout the trial.

- QTcF >500 msec.
- Change from baseline QTc > 60 ms.

Withdrawal decisions will be based on an average QTc value of triplicate ECGs. If an ECG demonstrates a prolonged QT interval, two more ECGs will be obtained over a brief period and the averaged QTc values of the three ECGs will be used to determine whether the subject must be discontinued from the trial.

9.8.3 Liver chemistry withdrawal criteria

Liver chemistry threshold stopping criteria have been designed to assure subject safety and to evaluate liver event aetiology. IMP administration will be stopped for any subject meeting any of the following liver chemistry stopping criteria, defined in the US Food and Drug Administration (FDA) guidance on drug-induced liver injury [15], and the subject will be withdrawn from the trial.

- Alanine aminotransferase (ALT) 3x upper limit of normal (ULN) and total bilirubin ≥ 2x ULN (> 35% direct bilirubin); or ALT 3x ULN and international normalised ratio (INR) > 1.5. Plasma bilirubin fractionation will be performed.
- ALT ≥ 5x ULN.
- ALT ≥ 3x ULN if associated with symptoms (new or worsening) believed to be related to hepatitis (*e.g.*, fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice), or believed to be related to hypersensitivity (*e.g.*, fever, rash or eosinophilia).

9.8.4 Renal function withdrawal criterion

IMP administration will be stopped for any subject meeting the renal function criterion below, and the subject will be withdrawn from the trial.

- Estimated glomerular filtration rate (eGFR) ≤ 30 mL/min/1.73 m^2 (determined by the revised Lund-Malmö GFR estimating equation).

9.8.5 Procedures for discontinuation of a subject from the trial

A subject who prematurely discontinues participation in the trial will always be asked about the reason(s) for discontinuation and the presence of any AEs. If a subject withdraws consent, the Investigator must ask the subject if he/she is willing, as soon as possible, to be assessed according to the procedures scheduled for the end-of-trial visit. Any ongoing AEs will be followed-up as described in Section 11.5.1.16.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF and final drug accountability must be performed. If the reason for discontinuation was an AE, the AE must be specified in the eCRF.

9.8.6 Subject replacement

Subjects who are prematurely withdrawn from the trial for any reason except the occurrence of AEs assessed as related to the IMP administration, may be replaced.

9.9 Randomisation

Not applicable.

9.10 Blinding

Not applicable.

9.11 Emergency unblinding during the trial

Not applicable.

10 TRIAL TREATMENTS

10.1 Identity of investigational medicinal products

The IMPs used in the trial are listed in Table 10.

10.2 Identity of auxiliary medicinal products

Not applicable.

10.3 Manufacturing, packaging, labelling and release

All manufacturing, packaging, labelling and release of IMP will comply with applicable good manufacturing practice (GMP) requirements [16].

The C21 capsules will be manufactured, packed, labelled, and released to qualified person (QP) by Ardena Gent NV, Mariakerke, Belgium. The C21 capsules will be packed in high-density polyethylene (HDPE) bottles with desiccant caps, containing 56 capsules per bottle. The C21 bottles will be packaged in outer cartons (secondary package). C21 will be shipped to the trial site by the trial pharmacy, Apoteket AB Clinical Trial Unit, Uppsala, Sweden.

On Day 6 of the trial, each subject will receive 1 tamper proof bottle of 56 C21 capsules for at-home administration from the evening of Day 6 and until the morning of Day 16. IMP administration compliance will be checked as described in Section 10.8.

The CYP and P-gp substrate drug products (caffeine, tolbutamide, midazolam, and nintedanib) are commercial drug product available within the EU (Table 10). The caffeine, midazolam and nintedanib drug products will be ordered from, labelled for the trial, and shipped to the trial site by the trial pharmacy, Apoteket AB Clinical Trial Unit, Uppsala, Sweden. The tolbutamide drug product will be imported from The Netherlands by ClinStorage AB, Solna, Sweden. It will be labelled for the trial and shipped to the trial site by the trial pharmacy.

10.4 Conditions for storage

The IMP will be stored in an access-controlled storage area at the trial site. The IMP will be stored at controlled room temperature, 15-25°C.

Temperature logs will be kept for the area where the IMP is stored. The temperature will be noted on a daily basis (working days only unless automatic temperature readings are available).

10.5 Preparation and accountability

IMP preparation will be done by trained personnel, *i.e.*, a site pharmacist or a registered nurse, in a dedicated room at the trial site.

The trial site and the Investigator will maintain a storage and accountability log as well as a drug dispensing log detailing the dates and quantities of trial medication received, prepared for and used by each subject, as well as trial medication returned or destroyed at the end of the trial. Any discrepancies between prepared and returned IMP must be explained and documented. Products deliberately and/or accidentally destroyed by the trial site or the subject must be accounted for.

Table 10 Investigational medicinal products

IMP	Type	Drug product and manufacturer	Formulation and strength	Dose and number of doses	SmPC
C21	Test/perpetrator drug	N/A	Oral capsules 50 mg	100 mg *BID* Day 4 to Day 18.	N/A
Caffeine	CYP1A2 substrate	Koffein Meda, 100 mg. Meda AB, Solna, Sweden.	Oral tablets 100 mg	Single dose 100 mg on Days 2, 5 and 18.	3
Tolbutamide	CYP2C9 substrate	Tolbutamide CF, 500 mg. Centrafarm, Breda, Netherlands.	Oral tablets 500 mg	Single dose 500 mg on Days 2, 5 and 18.	4
Midazolam	CYP3A4 substrate	Midazolam APL, 1 mg/mL. Apotek Produktion & Laboratorier AB, Stockholm, Sweden.	Oral solution 1 mg/mL	Single dose 2 mg on Days 2, 5 and 18.	5
Nintedanib	P-gp substrate	Ofev, 150 mg. Boehringer Ingelheim, Ingelheim am Rhein, Germany.	Oral soft capsules 150 mg	Single dose 150 mg on Days 1, 4 and 17.	6

10.6 Administration of investigational medicinal products

The IMP will be administered according to Table 10. From the evening of Day 6 until the morning of Day 16, subjects will self-administer C21 at home *BID* (morning and evening, a total of 20 doses). All IMP will be administered under fasting conditions and swallowed within a time span of 5 minutes with approximately 240 mL of tap water.

Subjects must fast overnight (at least 10 hours) prior to the anticipated morning IMP administrations on Day 1 (nintedanib), Day 2 (caffeine, tolbutamide, midazolam cocktail), Day 4 (C21 and nintedanib), Day 5 (C21 and caffeine, tolbutamide, midazolam cocktail), Day 17 (C21 and nintedanib), and Day 18 (C21 and caffeine, tolbutamide, midazolam cocktail), and until 4 hours post-dose. C21 should always be taken in a fasted state, *i.e.*, no food intake for at least 2 hours before and 1 hour after C21 intake. The latter applies to evening administrations of C21 in-clinic and to all at-home administrations. Water will be allowed *ad libitum* at the trial site except from 1 hour pre-dose to 1 hour post-dose.

10.7 Continued administration of investigational medicinal product

This is a phase 1 trial in healthy volunteers who will receive no medical benefit from the administration of IMP and thus there will be no continued administration of C21 or the substrates after the end of trial participation.

10.8 Administration compliance

During treatment visits to the trial site, the IMPs will be administered at the trial site under medical supervision to ensure compliance.

During at-home administration of C21, subjects will be contacted via telephone on Day 9 (±1 day) and Day 13 (±1 day) for a verbal confirmation of C21 self-administration and reminder of the importance of compliance with the planned dosing schedule and fasting conditions.

At Visit 3, the subjects will be asked to return any unused IMP and all empty IMP containers. A compliance count of the remaining C21 capsules will be performed by the trial site personnel.

10.9 Return and destruction of investigational medicinal product

Any unused trial medication will be returned to the Sponsor or destroyed at the trial site after approval from the Sponsor. Empty containers will be destroyed at the trial site. The Monitor will perform final IMP accountability reconciliation at the trial end to verify that all unused IMP is adequately destroyed and documented.

11 TRIAL ASSESSMENTS

The trial assessments are described in the sections below and the timing of assessments are detailed in the schedule of events (Table 3 to Table 9).

11.1 Recording of data

The PI will provide the Sponsor with all data produced during the trial from the scheduled assessments. They will ensure the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

It is important that blood sampling for PK, biomarker analysis and MIST occurs as close as possible to scheduled time. To achieve this, the timing priority order at a particular timepoint is:

- Blood sampling for PK, biomarker analysis and MIST
- Safety ECG
- Vital signs
- Clinical laboratory blood sampling

Timepoints for blood sampling, clinical laboratory sampling, safety ECG, and vital signs are outlined in in the schedule of events (Table 3 to Table 9). Time deviation allowances are detailed in Table 11.

For further details regarding the timing of blood sampling for PK, biomarker analysis and MIST, including time allowances, refer to Section 11.3.

11.2 Demographics and other baseline characteristics

11.2.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3. Consent for *CYP2C9* genotyping at the screening visit will be included in the main ICF.

There will be an additional, optional ICF for the exploratory genetic sampling (refer to Section 11.6.2).

11.2.2 Eligibility criteria

Eligibility criteria should be checked at the screening visit and verified before IMP administration on Day 1. The criteria are specified in Sections 9.4 and 9.5.

11.2.3 Demographic information

The following demographic data will be recorded: gender, age, ethnicity and race.

11.2.4 Height, weight and body mass index

Weight and height will be measured without shoes. Body mass index (BMI) will be calculated, with one decimal, from the recorded height and weight.

11.2.5 Medical/surgical history

Medical/surgical history will be obtained by subject interview to verify that the eligibility criteria are met.

The medical/surgical history must include all relevant diseases and surgeries within 2 weeks prior to the screening visit, as judged by the Investigator.

11.2.6 HIV and hepatitis B/C

Subjects will be tested for HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, hepatitis B virus surface antigen and hepatitis C virus antibodies prior to inclusion into the trial. Any positive result will exclude the subject from participating in the trial.

11.2.7 Urine drug screen and alcohol tests

Urine will be screened for drugs of abuse at timepoints outlined in the schedule of events (Table 3 to Table 9) using the Drug-Screen Multi-15 Dip Test (nal von minden GmBH or equivalent). Additional random tests can be performed during the trial period.

An alcohol test will be performed at timepoints outlined in the schedule of events (Table 3 to Table 9). Additional random tests can be performed during the trial period.

11.2.8 Physical examinations

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities. Complete physical examinations will be done at the screening visit (Visit 1) and at end-of-trial visit (Visit 4).

Symptom-driven physical examinations will be done upon discharge from the trial site on Day 6 and Day 19. A symptom-driven physical examination is only warranted when the subject has previously indicated any symptoms, or when the Investigator or site nurse has reason to believe there may be a problem and will only include the affected organ system or systems.

Any abnormalities will be specified and documented as clinically significant or not clinically significant in the eCRF. Abnormal post-IMP administration findings assessed by the Investigator as clinically significant will be reported as AEs.

11.2.9 Blood sampling for CYP2C9 genotyping

A venous blood sample (approximately 5 mL) will be drawn at the screening visit for genotyping of *CYP2C9*. Subjects with *CYP2C9* genotypes hetero- or homozygous for *CYP2C9*2* (Arg144Cys) and/or CYP2C9**3 (Ile359Leu)* variant alleles associated with altered CYP2C9 activity and tolbutamide metabolism will be excluded from trial participation [14] (see exclusion criterion no. 9).

CYP2C9 genotyping will be done by Clinical Chemistry and Pharmacology, Uppsala University Hospital, Uppsala, Sweden, on samples collected at screening, and results will be reviewed prior to subject inclusion.

11.2.10 Prior and concomitant medication

Prior medications taken within 2 weeks prior to first IMP administration will be obtained by subject interview to verify that the eligibility criteria are met (see also Section 11.2.10).

Medications are classified as prior if the stop date was before or on the day of the first dose administration (pre-dose) and as concomitant if ongoing on the day of the first dose administration, stopped after the first dose administration or started after the first dose administration. To distinguish between prior and concomitant medications on Day 1 (*i.e.*, the first day of IMP administration), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of prior/concomitant medication from the screening visit until the end-of-trial visit must be documented appropriately in the subject's eCRF. Relevant information (*i.e.*, name of medication, dose, dose form, unit, route, frequency, start and stop dates, reason for use) must be recorded. All changes in medication must be noted in the eCRF.

11.3 Blood sampling for pharmacokinetics, biomarker analysis and metabolites in safety testing

Venous blood samples (approximately 4 mL) will be collected through an indwelling venous catheter or by venepuncture at the pre-specified visits and time-points detailed in Table 4 to Table 9. Allowed deviations from planned sampling time points are detailed in Table 11 below. Blood sampling outside these time allowances will be considered protocol deviations. The date and time of collection of each sample will be recorded in the eCRF.

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged to separate plasma. The separated plasma from each blood sample will be divided into 5 aliquots in pre-labelled cryotubes (approximately 300 µL in each tube) and frozen at <-20°C within 1 hour of centrifugation. Further details will be described in a separate laboratory manual.

The aliquoted plasma samples will be used for the determination of plasma concentration and PK analysis (Section 11.4.1), biomarker analysis (Section 11.6.1), and MIST (Section 11.6.2).

Table 11 Blood sampling schedule

Sampling time points (hh:mm)[1]	Allowed time deviations
Pre-dose	-60 minutes[2]
00:15	±2 minutes
00:30	±3 minutes
00:45	±5 minutes
01:00	±6 minutes
01:30	±9 minutes
02:00	±12 minutes
03:00	±18 minutes
04:00	±24 minutes
06:00	±36 minutes
08:00	±48 minutes
10:00	±1 hour
12:00	±1 hour and 12 minutes[3]
16:00	±1 hour and 36 minutes
24:00 (Day 6 and Day 19)	±2 hours

1. Sampling time points as shown in Table 4 to Table 9. Time points at 24:00, 36:00, and 48:00 post-dose correspond to pre-dose, 12:00, and 24:00 (Day 6 and Day 19) post-dose time points, respectively, on the following days, and the corresponding time deviation allowances should be used.
2. Relative to anticipated morning IMP administration (00:00). All other allowed time deviations are relative to each planned time point.
3. On Day 4, Day 5, Day 17 and Day 18, the 12:00 samples must be collected prior to evening administration of C21 (second *BID* dose).

11.4 Assessments related to pharmacokinetic endpoints

11.4.1 Pharmacokinetic analysis

Plasma samples for the determination of plasma concentrations and PK characterization of C21, caffeine, tolbutamide, midazolam, nintedanib, as well as their metabolites (M1, paraxanthine, 4-hydroxy-tolbutamide, carboxy-tolbutamide, 1-hydroxy-midazolam, and BIBF 1202) after the administration of IMP will be analysed by Lablytica Life Science AB, Uppsala, Sweden, by means of a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The details of the analytical method used will be described in a separate bioanalytical report.

Other nintedanib metabolites may be analysed if considered appropriate.

11.5 Assessments related to safety endpoints

11.5.1 Adverse events

The PI is responsible for ensuring that all medical staff involved in the trial is familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding emergencies and phase 1 studies.

AEs will be handled in accordance with applicable regulations and guidelines [17, 18]. For the purpose of this trial, AEs will be assessed in relation to each IMP used in the trial, *i.e.*, C21, caffeine, tolbutamide, midazolam and nintedanib.

11.5.1.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment.

An AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

11.5.1.2 Definition of adverse reaction

An AR is any noxious and unintended response to a medicinal product related to any dose of the product.

The definition of an adverse reaction implies a reasonable possibility of a causal relationship between the AE and the IMP.

11.5.1.3 Definition of serious adverse event

An SAE is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening

  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the 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.
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Medical and scientific judgement 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 hospitalisation but may jeopardise 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 convulsion that do not result in hospitalisation; or development of drug dependency or drug abuse.

11.5.1.4 Definition of serious adverse reaction

The term serious adverse reaction (SAR) is used whenever either the Investigator or Sponsor or designee assesses that there is a reasonable possibility of a causal relationship between the SAE and the IMP.

11.5.1.5 Definition of suspected unexpected serious adverse reaction

A serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference safety information (Investigator's Brochure for an unapproved investigational medicinal product, Summary of Product Characteristics for an approved medicinal product used in accordance with the terms of the marketing authorisation).

11.5.1.6 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the signing of the ICF until the end-of-trial visit.

Any AE with start date on the day of the first IMP administration must be recorded with start time.

At the end-of-trial visit, information on new AEs or SAEs, if any, and stop dates for ongoing during events must be recorded as applicable.

Investigators will not be obliged to actively seek AEs or SAEs after conclusion of the trial participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the trial, and they consider the event to be reasonably related to the trial intervention or trial participation, the Investigator must promptly notify the Sponsor.

11.5.1.7 Collection of adverse events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject.
- AEs observed by the Investigator or medical personnel.
- AEs elicited based on non-leading questions from the Investigator or medical personnel.

11.5.1.8 Recording of adverse events

AEs must be recorded in the AE log of the eCRF. The Investigator must provide information on the AE, preferably as a diagnosis or at least as signs and symptoms; start and end dates, start and end time; intensity; causal relationship to IMPs, action taken, and outcome. Any AE with start date on the day of the first IMP administration must be recorded with start time. If the AE is serious, this must be indicated in the eCRF.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

11.5.1.9 Assessment of seriousness

The Investigator must assess and document the seriousness (serious or non-serious) of each AE using the definitions in Section 11.5.1.3. If the event is assessed as serious it must be reported as an SAE by the Investigator to the Sponsor according to Section 11.5.1.14.

For the seriousness criteria of inpatient hospitalisation or prolongation of existing hospitalisation to be fulfilled, the AE requires at least an overnight admission (24 hours) or prolongs a hospitalisation beyond the expected length of stay. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes.

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF, and that did not change in intensity, are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative approach will be taken, and the AE will be reported as an SAE.

11.5.1.10 Assessment of intensity

The grading of the intensity of AEs will follow the common terminology criteria for adverse events (CTCAE) v5.0 [19]. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the intensity of an AE using the following definitions, and record it in the AE Log of the eCRF:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self- care ADL**.

Grade 4 Life-threatening consequences: urgent intervention indicated.

Grade 5 Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc.*

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.5.1.11 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the use of each IMP, *i.e.*, C21, caffeine, tolbutamide, midazolam and nintedanib, using the definitions below. Each assessment should be recorded in the AE log of the eCRF.

Related There is a reasonable possibility that the AE was caused by the IMP. There is a reasonable time relationship to IMP intake. The AE cannot be explained by disease or other drugs. There may or may not be information about de-challenge or re-challenge. Disappearance of the AE upon de-challenge supports this category. Reappearance upon re-challenge is strongly supportive.

Not related	There is no reasonable possibility that the event was caused by the IMP. The temporal relationship to drug administration makes a causal relationship improbable or other drugs or underlying disease or conditions provide plausible explanations.
Not applicable	This assessment can be used, *e.g.*, in cases where the subject did not receive any IMP.

11.5.1.12 Outcome of adverse event

The Investigator must assess the outcome of an AE using the definitions below and record it on the AE log of the eCRF.

Recovered/resolved	The subject has recovered completely, and no symptoms remain.
Recovering/resolving	The subject's condition is improving, but symptoms still remain.
Recovered/resolved with sequelae	The subject has recovered, but some symptoms remain (*e.g.*, the subject had a stroke and is functioning normally, but has some motor impairment).
Not recovered/not resolved	The subject's condition has not improved, and the symptoms are unchanged (*e.g.*, an atrial fibrillation has become chronic).
Fatal	
Unknown	

11.5.1.13 Action taken with administration of investigational medicinal product

The Investigator must document the action taken with IMP administration using the definitions below and record it on the AE Log of the eCRF.

IMP not changed

IMP withdrawn

Not applicable

If the withdrawal of any of the IMPs (C21, caffeine, tolbutamide, midazolam or nintedanib) due to an AE is required, subject withdrawal should be considered as described in Section 9.8 following consultation with the Sponsor.

11.5.1.14 Reporting of serious adverse events

The Investigator must report SAEs within 24 hours of awareness to PrimeVigilance (refer to Section 5), this includes both initial information and any subsequent relevant/significant follow up information to a previously reported SAE. The primary mechanism for reporting an SAE will be via the paper SAE form provided in the investigator site file (ISF). The Investigator must fill in the SAE form, make a scanned copy, and send it by e-mail to PrimeVigilance. The trial site must notify the site Monitor via phone or e-mail about the submission of the SAE report.

All available information regarding the SAE must be entered in the AE log for the specific subject, *i.e.*, AE term, intensity, causality, outcome, seriousness criteria, action taken with trial drug, a narrative including the Investigators rationale for the causality assessment.

The SAE report will be reviewed by PrimeVigilance to ensure that the report is valid. PrimeVigilance will then acknowledge receipt of the SAE report to the reporting Investigator. For SAEs where important or relevant information is missing, follow-up queries to the site are raised promptly to keep the regulatory reporting timelines specified in Section 11.5.1.15.

On behalf of the Sponsor, PrimeVigilance will perform an independent assessment of causality, including a rationale for the assessment, which will be reviewed and approved by the Sponsor. The causality assessment given by the Investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator's causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report.

If any additional information or documentation (*e.g.*, autopsy report) on the SAE is required for PrimeVigilance's assessment of the SAE, PrimeVigilance will request this information from the Investigator, and the Investigator is required to promptly respond to the request.

Any subsequent relevant/significant follow up information to a previously reported SAE must be entered in the AE log for the specific subject. If the Investigator makes any changes to the assessment of the case *e.g.*, changes in seriousness, causality, or intensity, a justification for the change should be provided in the case narrative.

Detailed information on the SAE handling and SUSAR reporting will be described in a trial specific safety management plan (SMP).

11.5.1.15 Reporting of suspected unexpected serious adverse reactions

An SAE will be classified as a SUSAR when either the Investigator or the Sponsor assesses that there is a reasonable possibility of a causal relationship between the SAE and the IMP, and the Sponsor assesses the event as unexpected based on the applicable reference safety information (*i.e.*, in the IB/SmPC).

SAEs which are assessed by Sponsor to be a SUSAR will be reported by PrimeVigilance to the competent authority (CA) via EudraVigilance and to the IEC (if required) in accordance with local regulations and timelines:

- 7 calendar days if fatal or life-threatening.
- 15 calendar days if non-fatal and non-life-threatening.

The clock for expedited initial reporting (Day 0) starts as soon as the Sponsor or PrimeVigilance, has received the information containing the minimum reporting criteria. The date will be documented on the acknowledgement receipt to the reporting Investigator.

The Sponsor's trial physician is responsible for medical review of the SAE narrative in the Council for International Organisations of Medical Sciences (CIOMS) form (or equivalent) prior to expedited reporting.

The Sponsor or PrimeVigilance is responsible for informing the Investigators at the participating site of relevant information about potential SUSARs.

The Sponsor or a designee is responsible for once a year throughout the clinical trial (or on request), submit a safety report to the CA and the IEC taking into account all new available safety information received during the reporting period.

Detailed information on the SAE handling and SUSAR reporting are described in a trial specific SMP.

11.5.1.16 Treatment and follow-up of adverse events

Subjects with AEs that occur during the trial must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or to the end-of-trial visit, whichever comes first. At the end-of-trial visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs assessed as stable by the Investigator at the end-of-trial visit will not have to be followed up until resolution.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilised, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

11.5.1.17 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy of any female partners of male trial subjects, the outcome of the pregnancy (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even after the end of the subject's trial participation. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of the contraceptive medication.

All events of congenital abnormalities/birth defects are SAEs and must be handled and reported as such using the paper SAE form as described in Section 11.5.1.14. Spontaneous miscarriages should be reported and handled as AEs. All outcomes of pregnancy must be reported to the Sponsor and the PI on the pregnancy outcomes report form provided in the ISF.

11.5.1.18 Treatment of overdose

An overdose is a dose in excess of the dose specified in this clinical trial protocol (CTP).

In cases of accidental overdose of C21, standard supportive measures will be adopted as required. No known antidotes are available in case of overdose with C21. There have been no reported cases of C21 overdose.

In case of accidental caffeine overdose, standard supportive measures will be adopted as required, with special care to symptomatic treatment. The use of intravenous diazepam 5-10 mg should be considered in case of central nervous system excitation and/or seizures. In case of tachycardia and/or increased blood pressure, the use of a beta blocker should be considered. Antacids should be used as needed, complemented with the use of intravenous omeprazole 40 mg in case of hematemesis.

In case of accidental overdose with tolbutamide, standard supportive measures will be adopted as required, taking specific care to monitor the subject's blood glucose and providing standard treatment in case of hypoglycaemia. No specific antidotes are available in case of tolbutamide overdose.

In case of accidental overdose with midazolam, standard supportive measures will be adopted as required, with special care to symptomatic treatment of effects on the cardiovascular, pulmonary and central nervous systems. In case of a serious depression of the central nervous system, the use of the benzodiazepine antagonist flumazenil under careful observation of the subject should be considered.

In case of accidental overdose with nintedanib, standard supportive measures will be adopted as required. There are no known antidotes in case of nintedanib overdose.

Overdoses must be documented in the eCRF. An overdose with associated AE will be recorded as the AE diagnosis/symptoms in the AE log of the eCRF. An overdose without associated symptoms will only be reported in the subject's medical records and documented in the PD log.

11.5.2 Vital signs

Systolic and diastolic blood pressure and pulse rate will be measured in supine position after 10 minutes of rest.

Any vital signs outside of normal ranges will be judged as clinically significant or not clinically significant. The assessment will be recorded in the eCRF. Abnormal post-IMP administration findings assessed by the Investigator as clinically significant will be reported as AEs.

11.5.3 Safety electrocardiogram

Single 12-lead ECGs will be recorded in supine position after 10 minutes of rest using an ECG machine. The resting heart rate (HR) and PQ/PR, QRS, QT and QTcF intervals will be recorded.

ECGs will be reviewed and interpreted on-site by the Investigator.

Any abnormalities will be specified and documented as clinically significant or not clinically significant. Abnormal post-IMP administration findings assessed by the Investigator as clinically significant will be reported as AEs.

11.5.4 Clinical laboratory assessments

Blood samples for the analysis of clinical chemistry, haematology and coagulation parameters will be collected through venepuncture or an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital and analysed by routine analytical methods.

The clinical laboratory parameters are defined in Table 12 and will be assessed at visits and timepoints specified in Table 3 to Table 9.

Any laboratory values outside of normal ranges will be specified and documented as normal, abnormal not clinically significant, or abnormal clinically significant in the eCRF. Abnormal values assessed by the Investigator as clinically significant will be reported as AEs. If an abnormal value is associated with corresponding clinical signs or symptoms, the sign/symptom should be reported as the AE.

Table 12 Laboratory parameters

Category	Parameter
Clinical chemistry	Alanine aminotransferase (ALT)
	Albumin
	Alkaline phosphatase (ALP)
	Aspartate aminotransferase (AST)
	Bilirubin (total and conjugated)
	Calcium
	Creatinine (eGFR included)
	Glucose
	Potassium
	Sodium
	Urea
Haematology	Erythrocyte count
	Leukocyte count with differential count
	Haematocrit (B-EVF)
	Haemoglobin (Hb)
	Mean corpuscular volume (MCV)
	Mean corpuscular haemoglobin (MCH)
	Platelet count
Coagulation	Activated Partial Thromboplastin Time (APTT)
	Prothrombin Complex International Normalised Ratio (PK[INR])
FSH-test (at the screening visit, postmenopausal females only)	Follicle stimulating hormone (FSH)

eGFR: Estimated glomerular filtration rate.

11.6 Assessments related to exploratory endpoints

11.6.1 Biomarker analysis

Aliquots of plasma collected on Day 1 and Day 17 will be used for the analysis of the plasma concentration profiles of endogenous biomarkers of transporter inhibition and/or induction. The timepoints for biomarker sample analysis are specified Table 4 (Day 1) and Table 8 (Day 17). Biomarker plasma concentrations will be analysed by Lablytica Life Science AB, Uppsala, Sweden.

The primary exploratory biomarker will be coproporphyrin I, a biomarker of OATP1B activity, which will be assessed until 12 hours post-dose. Other exploratory biomarkers of transporter inhibition and/or induction may be analysed, including for longer than 12 hours, if considered appropriate.

Exploratory endpoints related to coproporphyrin I biomarker analysis will be reported in the CTR. Exploratory endpoints related to other biomarkers will not be reported in the CTR but may be included in a separate report.

11.6.2 Metabolites in safety testing

Aliquots of plasma collected on Day 17 (as detailed in Table 8) will be used for MIST analysis of C21 in plasma at steady state. A pre-IMP administration reference aliquot of plasma will be collected at pre-dose on Day 1 (as detailed in Table 4). Additional MIST analysis may be done on samples from other days, not only from Day 17, if considered appropriate.

MIST analyses will be done by MetaSafe Sweden AB, Södertälje, Sweden. Plasma samples for MIST analyses will be disposed of as detailed in Section 12.3 once analysed, and may be stored for a maximum of 10 years prior to analysis.

Exploratory endpoints related to MIST will not be reported in the CTR but may be included in a separate report.

11.6.3 Exploratory genetic blood sampling

Venous blood samples (approximately 5 mL) will be drawn for future genotyping of genes encoding enzymes and/or transporters of relevance for C21 and substrate metabolism and/or transport. This sampling is optional for the subjects and may be performed at any time during the trial.

A separate ICF will be prepared for this assessment as it is optional.

Exploratory endpoints related to genotyping will not be reported in the CTR but may be included in a separate report.

11.7 Appropriateness of measurements

All methods used are commonly used in standard medical care and in phase 1 clinical studies. Non-compartmental analysis of PK parameters is standard for phase 1 clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.3.

Blood sampling procedures for genotyping (*CYP2C9*), coproporphyrin I (exploratory biomarker for OATP1B1 inhibition) and exploratory genotyping are described in Sections 11.2.9, 11.6.1 and 11.6.2, respectively.

Blood and urine samples for safety parameters are collected according to standard procedures.

12.2 Volume of blood

The anticipated volume of blood samples collected during the trial from each subject will be approximately 428 mL (Table 13) over the full trial duration. For reference, a regular blood donation consists of between 350 mL to 450 mL (±10 %) for persons weighing at least 45-50 kg [20].

Table 13 Estimated blood volumes

Assessment	Estimated number of sampling occasions	Estimated volume per occasion	Total
Blood sampling for PK, biomarker analysis, MIST	81	4 mL	324 mL
HIV, Hepatitis B/C	1	4 mL	4 mL
CYP2C9 genotyping	1	5 mL	5 mL
Clinical laboratory profile (clinical chemistry, haematology, coagulation)	6	15 mL	90 mL
Exploratory genotyping (optional)	1	5 mL	5 mL
		Total:	**428 mL**

12.3 Handling, storage and destruction of laboratory samples

All biological samples will be registered in a biobank at CTC (Swedish Health and Social Care Inspectorate biobank registry number 893).

The samples for analyses of PK, biomarkers, MIST, and exploratory genotyping will be stored at ≤ -70 °C until analysed.

Any remains of the *CYP2C9* genotyping, clinical laboratory profile, as well as HIV and hepatitis B/C screening samples will be disposed of after analyses.

All plasma samples transferred to the Sponsor's biobank or to third party laboratories (including exploratory analyses) will, if not used, be disposed of after 10 years.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

The trial site keeps full traceability of collected biological samples from the trial subjects while in storage at the trial site until shipment and keeps documentation of receipt of arrival. The sample receiver (the analytical laboratory) will keep full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor will keep oversight of the entire lifecycle of the samples through internal procedures, monitoring of trial sites and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of/destroyed, if not already analysed and documented.

The PI will ensure that:

- Subject withdrawal of consent is notified immediately to the Sponsor.
- Biological samples from the subject, if stored at the trial site, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratories holding the samples are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the trial site and the action is documented.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical process, system and data identification

During CTP development, the Sponsor will identify those processes, systems (facilities, computerised systems) and data that are critical to ensure human subject protection and the reliability of trial results according to applicable SOPs and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) guideline [21].

Identified risks will be categorised separately from the CTP.

13.2 Quality assurance and quality control

The Sponsor has delegated the responsibilities outlined below to CTC whilst maintaining overall trial oversight:

- Implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs with regard to management of identified risks, CTP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.
- Securing agreements with involved subcontractors and to perform regular subcontractor oversight to ensure CTP compliance, GCP compliance and compliance with applicable regulatory requirements.
- Implementing a risk-based validated EDC system and maintain SOPs for the whole life-cycle of the system.
- QC application to each stage of data handling to ensure that all data are reliable and have been processed correctly.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the trial

The trial will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki [22] and are consistent with the ICH E6 (R2) guideline for GCP [21], the EU Clinical Trials Directive 2001/20/EC [23], and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The PI is responsible for submission of the CTP, the subject information and ICF, any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to applicable IEC for approval.

The Sponsor has delegated to CTC the responsibility to submit trial documents to the applicable CA according to local regulatory requirements.

Approval must be obtained in writing from both the IEC and CA before the first subject can be recruited.

The Sponsor will provide the CA, IEC and PI with safety updates/reports according to local requirements. Progress reports and notifications of SUSARs will be provided to the IEC according to local regulations and guidelines.

14.3 Subject information and consent

It is the responsibility of the Investigator or an authorised associate to give each potential trial subject verbal and written information before any trial specific assessments are performed.

The information will include the objectives and the procedures of the trial as well as any risks or inconvenience involved. It will be emphasised that participation in the trial is voluntary and that the subject may withdraw from participation at any time and for any reason, without any prejudice. All subjects will be given the opportunity to ask questions about the trial and will be given sufficient time to consider participation before signing the ICF.

Before performing any trial-related procedures the ICF must be signed and personally dated by the subject and by the Investigator. A copy of the subject information including the signed ICF will be provided to the subject.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information card and the signed ICF should be filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF must not be changed without approval from the Sponsor and the applicable IEC.

A separate ICF will be provided for the optional exploratory genotyping. Procedures for collection and documentation of this consent will follow the procedures described above for the consent for the main trial.

14.4 Subject information card

The subject will be provided with a subject information card including the following information:

- That they are participating in a clinical trial.
- Subject trial ID.

- That they are treated with the IMP.
- The name and phone number of the Investigator.
- The name and address of the Sponsor.

14.5 Subject privacy and data protection

The clinical personnel affirm and uphold the principle of the subject's right to privacy during and after the trial.

The ICF includes information that data will be recorded, collected and processed and information related to potential transfer to European Economic Area (EEA) or non-EEA countries. In accordance with the General Data Protection Regulation (GDPR [EU] 2016/679) [24], these pseudonymised data will not identify any persons taking part in the trial. If any part of the data is handled by any other organisation, inside or outside the European Union, appropriate agreements and/or other documentation will be established, to ensure that the data processing is performed in accordance with the provisions of the GDPR and other relevant legislation before any data transfer takes place.

The potential subject should be informed that by signing the ICF they approve that authorised representatives from the Sponsor and CTC, as well as the concerned IEC and CA, have direct access to their medical records for verification of clinical trial procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to their personal data and the right to request rectification of any data that is not correct and/or complete in accordance with GDPR [24] and the request will be raised to the PI.

The Investigator must file a subject identification list which includes sufficient information to link records, *i.e.*, the eCRF and clinical records. This list must be preserved for possible future inspections/audits but must not be made available to the Sponsor except for monitoring or auditing purposes.

Personal data that are collected in the trial such as health information and ethnicity are considered as sensitive personal data. This data will be pseudo anonymised, *i.e.*, personally identifiable information (PII) will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the trial. After the trial end, only pseudo anonymised data can be used, *i.e.*, aggregated data sets, can be used.

For this trial, the Sponsor is the data controller of all data processed during the trial (*e.g.*, trial master file [TMF], trial reports) and CTC AB is the data processor. Any subcontractors used in the trial are also data processors.

For data that are processed at the trial site (*e.g.*, medical records and ISF), CTC AB is the data controller.

14.6 Changes to the approved clinical trial protocol

Any proposed change to the approved final CTP, including appendices, will be documented in a written and numbered clinical protocol amendment. All substantial amendments to the protocol must be approved by the appropriate IEC and/or CA before implementation according to applicable regulations.

14.7 Audits and inspections

Authorised representatives of the Sponsor, a CA, or an IEC may perform audits or inspections at the trial site, including source data verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all investigation-related activities and documents, to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the CTP, ICH-GCP guidelines and any applicable regulatory requirements. The Investigator will contact the Sponsor immediately if contacted by a CA about an inspection at the trial site.

14.8 Insurance

Subjects will be covered under the Sponsor's liability insurance policy through the Swedish Pharmaceutical Insurance (Läkemedelsförsäkringen) (via CTC's share in the Swedish Pharmaceutical Insurance Service AB). The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable.

15 TRIAL MANAGEMENT

15.1 Training of trial site personnel

Before inclusion of the first trial subject, a Sponsor representative or delegate will perform a trial initiation visit at the trial site. The requirements of the CTP and related documents will be reviewed and discussed, and the investigational staff will be trained in any trial-specific procedures and system(s) utilised.

It is the responsibility of the Investigator to ensure that all personnel involved in the trial are fully informed of all relevant aspects of the trial and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this trial must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the trial together with their function and trial related duties delegated. A Curriculum Vitae will be available for all staff delegated trial-specific duties.

15.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all participating sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities.

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor and provided separately, the responsible Monitor will periodically visit the trial site at times agreed upon by the Investigator and the Monitor. At each monitoring visit, the role of the Monitor is (but not limited to) to:

- provide information and support to the investigational team.
- confirm that facilities and resources remain acceptable.
- confirm that the investigational team is adhering to the CTP, applicable SOPs, guidelines, manuals and regulatory requirements.
- verify that data are being accurately and timely recorded in the eCRFs and that IMP accountability checks are being performed.
- verify that data in the eCRF are consistent with the clinical records (SDV) in accordance with the RBM plan.
- verify that the correct informed consent procedure has been adhered to for participating subjects.
- ensure that withdrawal of informed consent to the use of the subject's biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject.
- verify that AEs are recorded and reported in a timely manner and according to the CTP.
- raise and escalate any serious quality issues, serious GCP breach and any data privacy breach to the Sponsor.

Centralised monitoring will also be performed continuously by project team members at the trial site in accordance with the RBM plan. When the trial has been completed, all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data documents

A separate origin of source data list will be generated before the start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the PI and the Monitor to confirm agreement before start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, and that verify the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the trial. They include laboratory notes, memoranda, material dispensing records, subject files, *etc*. The eCRF may constitute source data if clearly defined in the origin of source data list.

The Investigator must guarantee access to source documents to the Monitor, CAs and the IECs, if required.

15.4 Trial agreements

This trial is fully financed by the Sponsor, Vicore Pharma AB. The management and conduct of the clinical trial have been outsourced to the contract research organisation (CRO), CTC. The PI is an employee of CTC AB.

The agreements between Sponsor and CTC, and between Sponsor and the trial site, must be in place before any trial-related procedures can take place, or subjects be enrolled.

The Sponsor and CRO responsibility and duty split is regulated in a separate clinical trial agreement.

The PI must comply with all the terms, conditions, and obligations of the clinical trial agreement for this clinical trial.

15.5 Trial timetable and end of trial

The trial is expected to start in Q1 2023 and to be completed by Q2 2023.

A subject is considered to have completed the trial if they have completed all visits in the trial including the end-of-trial visit.

The end of the trial is defined as the date of the last visit of the last subject in the trial.

15.6 Termination of the trial

The Investigator or the Sponsor may terminate this trial prematurely for any reasonable cause. The IEC and CA must be informed promptly. Conditions that may warrant trial termination include, but are not limited to the following:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects included in the trial or potential trial subjects.
- A decision by the Sponsor to suspend or discontinue the development of the IMP.

If the CA obtains information that raises doubts about the safety or scientific validity of the trial, the CA may also suspend or prohibit the trial. Before the CA reaches its decision, it shall, except where there is imminent risk, ask the Sponsor and/or the Investigator for their opinion, to be delivered within one week (Directive 2001/20/EC, Article 12, Section 1) [23].

If the trial is prematurely terminated or suspended for any reason, the Investigator/institution must promptly inform the trial subjects and must assure appropriate follow-up for the subjects.

15.7 Reporting and publication

15.7.1 Clinical trial report

A summarising report will be submitted to a publicly available database (EudraCT) within 12 months after completion of the trial, in accordance with applicable regulations.

After completion of the trial, an ICH E3 [25] guideline-compliant CTR describing the conduct of the trial, any statistical analyses performed, and the results obtained will be prepared by the Sponsor or their designee. The CTR will be reviewed and approved by, as a minimum, the PI and the Sponsor.

Results obtained from exploratory analyses may be reported separately.

15.7.2 Annual safety report

If the trial duration exceeds one year, the Sponsor must submit development safety update report (DSUR) to the CA and to the IEC. The report must summarise all pertinent safety information collected during the reporting period and contain an update of the risk-benefit evaluation if there has been any change since the approval of the clinical trial.

15.7.3 Confidentiality and ownership of trial data

Any confidential information relating to the IMP or the trial, including any data and results from the trial, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the trial are responsible for protecting the confidentiality of this proprietary information.

15.7.4 Publication

The results from this trial may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The PI is responsible for maintaining essential documents, (as defined in ICH E6(R2), Section 8 [21]) for 15 years after finalisation of the CTR. This includes any original source documents related to the trial, the subject identification list (providing the sole link between named subject source records and pseudonymous eCRF data), the original signed ICFs and detailed records of IMP disposition.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the TMF in accordance with the ICH E6(2) guideline, Section 8 [21], and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the site and filed in the investigator trial file for archiving for 15 years after finalisation of the CTR.

The completed original eCRFs are the sole property of the Sponsor and must not be made available in any form to third parties, except for authorised representatives of appropriate health/regulatory authorities, without written permission from the Sponsor.

16 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database set-up and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the CTP.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of computerised online edit checks identifying *e.g.*, data values that are outside the allowed range and SAS-programmed batch checks on data exports. All trial-specific and standard data validation programming will be tested prior to being used on final data.

Detailed information on data management will be described in a trial-specific Data Management Plan (DMP).

16.1 The web-based eCRF

Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by Viedoc Technologies AB. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or at bedside (if the eCRF data constitutes source data). Source data are to be defined at the site before inclusion of the first subject (Section 15.3).

Authorised site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorised trial site personnel prior to the trial being initiated and any data being entered into the system for any trial subject.

16.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other project team member besides site staff can enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that preferably same individual who made the initial baseline determinations completes all corresponding follow-up evaluations. The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable or unknown, the Investigator or assigned clinical staff will record such information in the eCRF. The Investigator will be required to electronically sign off on the clinical data. This will be performed by means of the Investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature.

16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there are no discrepancies for critical data as described in the RBM plan. All entries, corrections, and alterations are to be made by the Investigator or designee.

If corrections are needed, queries will be raised within the eCRF, either as a result of built-in edit checks or manually raised by the Monitor. An appropriate member of the site staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query.

16.4 Audit trail

All entries in the eCRF will be fully recorded in a protected audit trail. Once clinical data have been saved, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged.

16.5 External data

External data consists of data that are not recorded in the eCRF. Data may be received in electronic format or as a paper printout. Key variables are defined to uniquely identify each sample record. File and data formats are agreed with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at the trial site. AEs and medical/surgical history verbatim terms are coded using the medical dictionary of regulatory activities (MedDRA, latest version available at eCRF setup).

Prior and concomitant medications will be coded according to the World Health Organisation (WHO) drug dictionary classification system WHODrug.

All coding will be approved by the Sponsor prior to database lock.

16.7 Database lock

When all data have been entered and discrepancies solved, clean file will be declared, the database will be locked, and the data will be analysed.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate Statistical Analysis Plan (SAP), which will be signed and approved prior to database lock.

The endpoint analyses will be performed by CTC, except future analyses of exploratory endpoints after completion of the CTR.

17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum and maximum value. For AUC and C_{max} parameters, the geometric mean and geometric coefficient of variation (CV%) will be presented.

Categorical data will be presented as counts and percentages. When applicable, summary data will be presented by treatment, and by assessment time. Individual subject data will be listed by subject number, treatment, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC). The PK parameters will be calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 8.3 or later (Certara, U.S.A.).

Baseline will be defined as the last non-missing data collection point prior to the first administration of IMP. All hypothesis testing will use a significance level of 5 %. No imputation of missing data will be performed.

17.2 Determination of sample size

No formal sample size calculation has been performed for this trial. The proposed sample size is considered sufficient to provide adequate information to meet the trial objectives.

17.3 Analysis data sets

Table 14 Definitions of analysis sets

Analysis set	Definition
Full analysis set (FAS)	All included subjects who have received at least 1 dose of any IMP and provided at least 1 post-baseline data point. This analysis set will be used for safety assessments.
PK analysis set (PKAS)	All included subjects who received at least 1 dose of IMP and provided an evaluable plasma concentration profile, and who have no AEs or protocol deviations judged to affect the PK analysis. Individual PK values may be excluded from the analysis as specified in the SAP.
PK ratio analysis set (PKRAS)	All subjects who completed the trial up until at least Day 19 and provided evaluable plasma concentration profiles, and who have no AEs or protocol deviations judged to affect the PK analysis. Individual PK values may be excluded from the analysis as specified in the SAP.

17.4 Description of trial population

17.4.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight, height and BMI will be presented for all subjects. All data will be listed by subject.

17.4.2 Medical/surgical history and prior/concomitant medication

Medical/surgical history will be presented by system-organ-class (SOC) and preferred term (PT). Prior/concomitant medications will be presented by the WHO anatomical therapeutic chemical (ATC) classification level 4 and WHODrug preferred names.

All data will be listed by subject.

17.4.3 Administration compliance

All administered individual doses will be listed by subject.

17.5 Analysis of primary endpoints

17.5.1 Analysis of pharmacokinetics

The PK analysis will be performed by CTC. PK parameters will be calculated based on the PKAS by standard non-compartmental analysis (NCA) using the software Phoenix WinNonlin® version 8.3 or later (Certara Inc, USA).

The primary PK evaluation is to evaluate the impact of C21 on the PK of caffeine, tolbutamide, midazolam, nintedanib and their primary metabolites. PK parameters to be calculated for caffeine, tolbutamide, midazolam, nintedanib and their primary metabolites are C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{inf}. Additional parameters may be calculated if data permits and considered appropriate. The ratios of C_{max}, $AUC_{0\text{-}last}$ and AUC_{inf} with C21 and without C21 will be calculated based on the PKRAS.

The secondary PK evaluations are to evaluate the PK of C21 and its main metabolite M1. PK parameters to be calculated for C21 and M1 are C_{max}, T_{max}, $AUC_{0\text{-}last}$ and AUC_{tau}. Additional parameters may be calculated if data permits and considered appropriate.

For AUC_{inf}, the area under the plasma concentration *vs.* time curve will be calculated to the timepoint of the last quantifiable plasma concentration of respective IMP and then extrapolated to infinity using the concentration in the last quantifiable sample and the estimated terminal elimination rate constant ($Lambda_z$).

In general, PK data will be presented by substance (C21, caffeine, tolbutamide, midazolam, nintedanib and their main metabolites) using summary statistics with number of measurements, arithmetic mean, SD, as well as median, minimum and maximum values. For AUC and C_{max} parameters, the geometric mean and geometric CV% will be presented. C_{max} and AUC ratios will be analysed using a paired t-test and summarised by substance using geometric means and their 90% confidence intervals.

All data will be listed by subject.

17.6 Analysis of secondary endpoints

17.6.1 Secondary pharmacokinetic endpoints

Refer to Section 17.5.1.

17.6.2 Adverse events

An overview of all AEs, including SAEs, intensity, relationship to IMP and deaths will be presented. The incidence of AEs and SAEs will be summarised by SOC and PT by treatment and overall. All AE data will be listed by subject and include the verbatim term entered by the Investigator.

17.6.3 Vital signs

Vital signs will be summarised by trial day. Data will be presented with absolute change from baseline. All data will be listed by subject.

17.6.4 Electrocardiogram

All ECGs will be categorised as ”normal”, ”abnormal, not clinically significant”, or ”abnormal, clinically significant” (as judged by the Investigator) and summarised by trial day using frequency tables. Changes over time will be presented using shift tables, if appropriate. All data will be listed by subject.

17.6.5 Clinical laboratory

Clinical laboratory data will be summarised by trial day with absolute change from baseline. Abnormal, clinically significant values will be summarised separately, if considered appropriate. All data will be listed by subject.

17.7 Analysis of exploratory objectives

Biomarker data related to coproporphyrin I will be summarised by trial day with absolute change from baseline. All data will be listed by subject.

The analysis of other exploratory endpoints, including other biomarkers, MIST and exploratory genotyping, will not be reported in the CTR. The analyses related to these exploratory endpoints will be specified elsewhere and may not be included in the SAP.

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19 SIGNATURES

19.1 Principal Investigator statement

I, the undersigned, have read and understood this CTP and agree to conduct the trial accordingly and to comply with the Investigator obligations stated in this CTP, GCP and applicable regulatory requirements.

Principal Investigator

19.2 Approval of the clinical trial protocol

I, the undersigned, approve this CTP.

Sponsor signatories

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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