Clinical Trial Protocol

Title:	**A randomized, double-blind, placebo-controlled, 2-way cross-over trial evaluating the effect of C21 on endothelial dysfunction and safety in subjects with type 2 diabetes mellitus**
Short Title:	**A trial to evaluate the effect of C21 on endothelial dysfunction in subjects with type 2 diabetes**
Sponsor:	**Vicore Pharma AB** **Kornhamstorg 53** **SE-111 27 Stockholm** **Sweden**
Trial ID:	**VP-C21-013**
EudraCT No.:	**2023-000168-77**
Investigational Medicinal Products:	**C21**
Indication:	**Type 2 Diabetes Mellitus (T2DM)**
Phase:	**1b**
Version:	**2.0**
Date:	**03-MAR-2023**

Sponsor's Approval of Clinical Trial Protocol

This trial protocol was subject to critical review by the sponsor. The information it contains is consistent with current knowledge of the risks and benefits of the investigational medicinal product, as well as with the ethical and scientific principles governing clinical research as set out in the latest version of the Declaration of Helsinki and the current International Conference on Harmonisation (ICH) Guideline for GCP.

This trial will be conducted in compliance with the protocol, GCP, and applicable regulatory requirements.

Sponsor's Medical Expert:

[redacted]

Vicore Pharma AB

E-mail:

[redacted]

[redacted]

_____

Signature

In the case of a medical emergency, the Investigator may contact the Sponsor's Medical Expert.

Sponsor's Statistical Expert:

[redacted]

[redacted]

_____

Signature

VP Clinical Development:

[redacted]

[redacted]

_____

Signature

Signatory Investigator's Approval of Clinical Trial Protocol

This trial will be conducted in compliance with the protocol, GCP, and the applicable regulatory requirements.

I confirm that I agree to conduct this trial in compliance with the Declaration of Helsinki, the current International Conference on Harmonisation (ICH) Guideline for GCP and applicable regulatory requirements.

Furthermore, I confirm that I have read and understood the present clinical trial protocol and agree to conduct the trial in compliance with this. I fully understand that any changes from the clinical trial protocol constitute a deviation which will be notified to sponsor.

Coordinating Investigator:

Prof Jan Nilsson

Clinical Research Centre 60:13

Jan Waldenströms gata 35

SE-214 28 Malmö

Phone: [redacted]

_____

Signature

TABLE OF CONTENTS

1 PROTOCOL SUMMARY

Trial Title	A randomized, double-blind, placebo-controlled, 2-way cross-over trial evaluating the pharmacodynamic effect of C21 on endothelial dysfunction and safety in subjects with type 2 diabetes mellitus	
Trial ID	VP-C21-013	
Trial Phase	1b	
Objectives and Endpoints	**Objectives**	**Endpoints**
	Primary	
	To evaluate the pharmacodynamic effect of a single oral dose of 200 mg C21 versus placebo on endothelial dysfunction in subjects with type 2 diabetes mellitus (T2DM)	• Reactive hyperemia index (RHI) score as measured by EndoPAT
	Secondary	
	[redacted]	[redacted]
Trial Design	This is a randomized, double-blind, placebo-controlled, 2-way cross-over trial to evaluate the pharmacodynamic effect of C21 on endothelial dysfuction and safety in subjects with T2DM. The subjects will have 4 visits at the trial site: • **Visit 1:** Screening will take place within 3 weeks of the planned first IMP administration (Day -21 to Day -1) and will include the subject's signing of the informed consent and an eligibility check. • **Visits 2 and 3:** Subjects will visit the trial site for single-dose, oral IMP administration (C21 or placebo according to randomization list), safety and pharmacodynamic efficacy (EndoPAT) assessments. There will be a washout period of 3 to 14 days between visits 2 and 3. • **Visit 4:** A final end-of-trial visit will take place 5 to 14 days after Visit 3 or after early withdrawal from trial.	
Number of Subjects	The trial will include 10 to 14 randomised subjects, with 12 being the target number.	
Inclusion Criteria	For inclusion in the trial, subjects must fulfil the following criteria: 1) Written informed consent, consistent with ICH-GCP R2 and local laws, obtained before the initiation of any trial-related procedure. 2) Male or female patient aged ≥ 40 years at the time of the screening visit (Visit 1). 3) Documented diagnosed with T2DM prior to the screening visit (Visit 1). 4) An RHI score ≤2 as assessed by EndoPAT at the time of the screening visit (Visit 1).	

	5) Contraceptive use by men and women of childbearing potential which is highly effective and consistent with local regulations regarding the methods of contraception for those participating in clinical trials.
Exclusion Criteria	Subjects must not enter the trial if any of the following exclusion criteria are fulfilled: 1) Concurrent serious medical condition which in the opinion of the investigator constitutes a risk or a contraindication for the participation in the trial or that could interfere with the trial objectives, conduct or evaluation. 2) Known, active hepatitis B, C, or human immunodeficiency virus (HIV) infection (*i.e.,* HIV with a CD4 count <500 cells/mm^3). 3) Impaired hepatic function or clinically significant liver disease, which in the investigator's opinion makes the subject inappropriate for this trial. 4) Severe renal impairment (*i.e.,* estimated glomerular filtration rate (eGFR) ≤30 mL/min/1.73 m^2). 5) Prolonged QTcF (QT interval with Fridericia's correction) (>450 ms), atrial fibrillation, clinically significant arrhythmia or other clinically significant abnormality in the resting ECG at screeening (Visit 1), as judged by the investigator. 6) Unstable or deteriorating cardiac condition. 7) 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. 8) Pregnant or breast-feeding female subjects. 9) Malignancy within the past 5 years with the exception of in situ removal of basal cell carcinoma and cervical intraepithelial neoplasia grade I. 10) Planned major surgery within the duration of the trial. 11) Treatment with the medications listed below within 14 days prior to screening (Visit 1) or anticipated need for such medication during the participation in this trial: • Insulin. • Glucagon-like peptide-1 (GLP-1) agonists. • Strong Cytochrome P450 (CYP) 3A4 inducers (*e.g.,* rifampicin, phenytoin, carbamazepine and St. John's Wort). • Strong CYP 3A4 inhibitors (*e.g.* clarithromycin, ketoconazole, nefazodone, itraconazole, ritonavir). • Warfarin, digoxin and other medication where the dose needs to be individually titrated. • Narrow therapeutic index drugs that are substrates of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or OATP1B1. 12) Current or previous participation in any other clinical trial where the subject has received a dose of IMP within 1 month or 5 half-lives of the IMP, whichever is longest, prior to screening. 13) Abnormal laboratory value at screening indicating a potential risk for the subject if enrolled in the trial as evaluated by the investigator.
Investigational medicinal product, dosage and mode of administration	<u>C21</u> C21 is supplied as a 50 mg capsule for oral administration. C21 200 mg (4 capsules) will be administered as a single oral dose. <u>Placebo to C21</u>

	Placebo to C21 is supplied as a capsule for oral administration, manufactured as C21 without the active pharmaceutical ingredient. Placebo to C21 (4 capsules) will be administered as a single oral dose.
[redacted]	
Criteria for Evaluation	All subjects who have received at least one dose of IMP will be included in the safety analyses. The pharmacodynamic analyses (assessed by EndoPAT) will include all treated subjects for whom the pharmacodynamic parameters can be calculated.
Statistical Analyses	All quantitative variables will be summarized by assigned treatment and time point using standard descriptive statistics. Qualitative variables will be summarized by assigned treatment and time point by means of absolute and relative frequencies. Percentage change in RHI will be compared between treatments using an analysis of variance model adjusting for treatment, period, and subject (random).
Sample Size Calculation	Sample size is based on what is usually seen in this type of trial and not on any statistical assumptions.
Trial Reporting	After completion of the trial, an International Council for Harmonisation (ICH) E3 guideline-compliant clinical trial report (CTR) will be prepared.

2 FLOW CHART

Table 1 Flow Chart for Trial Procedures

	CTP section	Screening	Trial Intervention Visits		Follow-up/end-of-trial[1]
		Visit 1	Visit 2	Visit 3	Visit 4
		Day -21 to Day -1	Day 1	3 to 14 days after Visit 2	5-14 days after Visit 3
Informed consent	8.1.1	x			
Eligibility criteria	8.1.2	x	x[2]	x[2]	
Demographics	8.1.3	x			
Height and weight	8.1.4	x			
Medical/surgical history	8.1.5	x			
12-lead ECG	8.1.6	x	x[3]	x[3]	x
Pharmacodynamic (EndoPAT) assessment[4]	8.2.1	x	x	x	
Adverse events[5]	8.3.1	----------x----------			
Pregnancy test (urine dipstick)[6]	8.3.2	x	x	x	x
Physical examination[7]	8.3.3	x	x	x	x
Vital signs	8.3.4	x	x	x	x
Laboratory safety assessment	8.3.5	x			x
FSH[8]	8.3.5	x			
Prior and concomitant medication	8.4.1	----------x----------			
Randomisation	7.3		x		
IMP administration[9]	7.4		x	x	

BMI=body mass index, CTP=clinical trial protocol, ECG=electrocardiogram, FSH=follicle stimulating hormone, IMP=investigational medicinal product.

1. Or after early withdrawal.
2. Confirmation of relevant eligibility criteria.
3. At Visits 2 and 3, a 12-lead ECG will be collected within 60 minutes prior to IMP administration and before initiation of the EndoPAT assessment, 50±10 minutes after the IMP administration.
4. At Visits 2 and 3, the EndoPAT assessment will be performed 50±10 minutes after the IMP administration.
5. Adverse events will be recorded from signing informed consent until end of trial (Visit 4).
6. Applicable only in women of childbearing potential. If positive, the urine dipstick will be followed-up by a blood test.
7. A physical examination will be done at Visits 1 and 4. Symptom-driven physical examination will be done upon admission and discharge from the trial site at Visits 2 and 3.
8. Applicable only in postmenopausal females.
9. C21 or placebo will be administered at Visits 2 and 3, respectively, according to the randomisation list.

3 BACKGROUND AND RATIONALE

3.1 Endothelial Dysfunction

The endothelium is a monolayer of cells that lines all blood vessels, and which comprises the systemic and lymphatic capillaries. The vascular endothelium is an active paracrine, endocrine, and autocrine organ that regulates the vascular tone and maintains vascular homeostasis.

The basic mechanisms involved in atherogenesis indicate that deleterious alterations of endothelial physiology, otherwise known as endothelial dysfunction, represent a key early step in the development of atherosclerosis and are also involved in plaque progression and the occurrence of atherosclerotic complications.

Endothelial dysfunction is the single most important factor for increased cardiovascular risk in patients with type 2 diabetes mellitus (T2DM).

3.2 Current Treatment of Endothelial Dysfunction

Non-pharmacological therapies involve diet control and exercise. Pharmacological treatment of patients with T2DM and endothelial dysfunction includes metformin, peroxisome proliferator-activated receptor–γ (PPAR-γ) agonists and concomitant diseases are treated to reduce blood pressure and cardiovascular risk factors.

3.3 Compound 21

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 idiopathic pulmonary fibrosis (IPF).

C21 has been granted orphan drug designation in IPF by the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA).

3.3.1 Non-clinical Data

[redacted]

[redacted]

3.3.2 Clinical Experience

[redacted]

3.4 Rationale for Trial

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.

The renin angiotensin system (RAS) is intimately involved in cardiovascular disease where stimulation of the angiotensin II type 1 (AT1) receptor leads to increased blood pressure and, consequently, increased risk of cardiovascular events. The angiotensin II type 2 (AT2) receptor

has an opposite effect and in animal studies it has been shown that stimulation protects against cardiovascular risk in various diabetes models (Steckelings et al., 2022). Endogenous AT2R activation is critically involved in the control of vascular tone by evoking vasodilation in response to flow (shear stress) that in turn opposes active myogenic tone. This flow-mediated increase in vessel diameter, mediated by endothelial-derived NO, is best described for AT2R in the mesenteric circulation (Matrougui et al., 1999).

[redacted]

3.5 Rationale for Trial Design

This is a randomised, double-blind, placebo-controlled, 2-way cross-over trial to evaluate the effect of C21 on endothelial dysfunction and safety in subjects with T2DM.

A cross-over design is applied to control for inter-individual variability.

Randomisation is applied to minimise bias in the assignment of subjects. Double-blinded treatment is applied to reduce potential bias during data collection and evaluation of endpoints.

The timing of the IMP administration in relation to the EndoPAT assessment is based on the pharmacokinetic properties of C21.

Overall, the trial will provide important data to support the design of further clinical trials.

3.6 Rationale for Dose and Dosing Regimen

A single dose of 200 mg C21 is selected to achieve a sufficient coverage of the AT2 receptor [redacted]

3.7 Risk-Benefit Assessment

The subjects participating in this trial will have no medical benefit from participation and their safety and wellbeing are of outmost importance.

[redacted]

dose of 200 mg C21, has been evaluated in a Phase 1 trial in healthy subjects (C21-003) and a

Aside from the risks related to C21, as detailed above, there may also be risks related to trial-specific procedures such as the EndoPAT assessment. EndoPAT is a non-invasive method to assess endothelial dysfunction. During the assessment, a blood-pressure cuff is used to achieve and maintain arterial occlusion for 5 minutes. Inflating the cuff might cause some stress and discomfort to the patient, however, the risk associated with its use is considered low and ethically justifiable. Other trial-specific evaluations and sampling procedures, such as blood-pressure measurements using a blood pressure cuff and blood sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable. Overall, the combined

4 OBJECTIVES AND ENDPOINTS

Primary objective	Endpoints	Assessments	Analysis
To evaluate the pharmacodynamic effect of a single oral dose of 200 mg C21 versus placebo on endothelial dysfunction in subjects with T2DM	• RHI score as measured by EndoPAT.	EndoPAT assessment (Section 8.2.1).	Section 13.5
Secondary objectives	**Endpoints**	**Assessments**	**Analysis**
To evaluate the safety of a [redacted]	[redacted]	[redacted]	[redacted]
[redacted]	AI score as measured by EndoPAT.	EndoPAT assessment (Section 8.2.1).	Section 13.5

5 TRIAL POPULATION

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

5.1 Recruitment

Subjects will be recruited from the trial site's trial database.

5.2 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.

A screening number 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.

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 informed consent form (ICF) and reason(s) for screening failure.

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

5.3 Inclusion Criteria

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

1) Written informed consent, consistent with ICH-GCP R2 and local laws, obtained before the initiation of any trial-related procedure.
2) Male or female patient aged ≥ 40 years at the time of the screening visit (Visit 1).
3) Documented diagnosed with T2DM prior to the screening visit (Visit 1).
4) An RHI score ≤2 as assessed by EndoPAT at the time of the screening visit (Visit 1).
5) Contraceptive use by men and women of childbearing potential which is highly effective and consistent with local regulations regarding the methods of contraception for those participating in clinical trials.

5.4 Exclusion Criteria

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

1) Concurrent serious medical condition which in the opinion of the investigator constitutes a risk or a contraindication for the participation in the trial or that could interfere with the trial objectives, conduct or evaluation.

2) Known, active hepatitis B, C, or human immunodeficiency virus (HIV) infection (*i.e.,* HIV with a CD4 count <500 cells/mm^3).
3) Impaired hepatic function or clinically significant liver disease, which in the investigator's opinion makes the subject inappropriate for this trial.
4) Severe renal impairment (*i.e.,* estimated glomerular filtration rate (eGFR) ≤30 mL/min/1.73 m^2).
5) Prolonged QTcF (QT interval with Fridericia's correction) (>450 ms), atrial fibrillation, clinically significant arrhythmia or other clinically significant abnormality in the resting ECG at screeening (Visit 1), as judged by the investigator.
6) Unstable or deteriorating cardiac condition.
7) 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.
8) Pregnant or breast-feeding female subjects.
9) Malignancy within the past 5 years with the exception of in situ removal of basal cell carcinoma and cervical intraepithelial neoplasia grade I.
10) Planned major surgery within the duration of the trial.
11) Treatment with the medications listed below within 14 days prior to screening (Visit 1) or anticipated need for such medication during the participation in this trial:
    - Insulin.
    - Glucagon-like peptide-1 (GLP-1) agonists.
    - Strong Cytochrome P450 (CYP) 3A4 inducers (*e.g.,* rifampicin, phenytoin, carbamazepine and St. John's Wort).
    - Strong CYP 3A4 inhibitors (*e.g.,* clarithromycin, ketoconazole, nefazodone, itraconazole, ritonavir).
    - Warfarin, digoxin and other medication where the dose needs to be individually titrated.
    - Narrow therapeutic index drugs that are substrates of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP3A4, or OATP1B1.
12) Current or previous participation in any other clinical trial where the subject has received a dose of IMP within 1 month or 5 half-lives of the IMP, whichever is longest, prior to screening.
13) Abnormal laboratory value at screening indicating a potential risk for the subject if enrolled in the trial as evaluated by the investigator.

5.5 Restrictions During the Trial

Subjects must be willing to comply with the restrictions as outlined in Sections 5.5.1 and 5.5.2.

5.5.1 General Restrictions

1. Contraception requirements: Women of child bearing potential (WOCBP) must practice abstinence (if that is their preferred lifestyle) from Visit 1 to Visit 4, or must agree to use a highly effective method of contraception with a failure rate of <1% to prevent pregnancy (combined [oestrogen and progestogen containing] hormonal contraception associated with inhibition of ovulation [oral, intravaginal, transdermal], progestogen-only hormonal contraception associated with inhibition of ovulation [oral, injectable, implantable], intrauterine device [IUD] or intrauterine hormone-releasing system [IUS]) from at least 4 weeks prior to the first IMP administration until 4 weeks after last IMP administration. Their male partner must agree to use a condom during the same time frame, unless he has had a demonstrated successful vasectomy more than 6 months ago.

   Males should use condom and their female partner of child-bearing potential must use a contraceptive method with a failure rate of <1% to prevent pregnancy (see above) and drug exposure of a partner and refrain from donating sperm from the date of dosing until 3 months after the last IMP administration.
2. Meals and dietary restrictions: Subjects must fast for at least 3 hours prior to admission to the trial site for Visits 2 and 3, and until end of the EndoPAT assessment. Water is allowed *ad libitum,* except for 1 hour before and 1 hour after IMP administration.
3. Alcohol: Consumption of alcohol is not allowed from 12 hours prior to admission to the trial site for Visits 2 and 3, and during these visits.
4. Coffee, tea, and other caffeine-containing beverages: No coffee, tea or other caffeine-containing beverages are allowed from 2 hours prior to admission to the trial site for Visits 2 and 3, and until end of the EndoPAT assessment.
5. Taurine-containing beverages: Beverages containing taurine, *e.g.,* energy drinks such as Red Bull and Monster Energy, are not allowed from 2 hours prior to admission to the trial site for Visits 2 and 3, and until the end of the EndoPAT assessment.
6. Nicotine: Smoking or the use of nicotine-containing products, including non-tobacco oral nicotine products, is not allowed from 3 hours prior to admission to the trial site for Visits 2 and 3, and until end of the EndoPAT assessment.
7. Grapefruit and grapefruit-containing products: The consumption of grapefruit and/or grapefruit-containing products such as jams, jellies, preserves, and fruit juices is not allowed from 12 hours prior to until 12 hours after IMP administration at Visits 2 and 3.
8. Exercise: Subjects must refrain from strenuous exercise, defined as greater than 70% of the maximal pulse rate for 1 hour or more, for at least 12 hours prior to admission to the trial site for Visits 2 and 3, and until end of the EndoPAT assessments.
9. Blood donation: 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.
10. Participation in other clinical trials: Subjects are not allowed to participate in any other interventional clinical trial from the screening visit until the end-of-trial visit.

5.5.2 Prior and Concomitant Medication

Concomitant medication is defined as any medication or vaccine (including over-the-counter, prescription medicines, recreational drugs, vitamins, and/or herbal supplements) or other specific categories of interest that the subject is receiving at the time of enrolment or receives during the trial.

All prior and concomitant medication taken up to 2 weeks prior to screening (Visit 1) and until the follow-up visit (Visit 4) should be recorded.

Prior and concomitant medication must be recorded along with:

- Reason for use.
- Dates of administration including start and end dates.
- Dosage information including dose and frequency.

The medical monitor should be contacted if there are any questions regarding prior and concomitant medication.

The concomitant medication withholds requirements presented in Table 2 must be evaluated at screening (Visit 1) and prior to performing the EndoPAT at Visits 2 and 3. Subjects violating any of the requirements can be re-scheduled for a new visit within less than 10 days. Re-scheduling can only occur once per visit. Withholding medication should only be done if clinically acceptable based on investigator's judgement.

Table 2. Concomitant medication withholds requirements

Time to withhold	Therapy/substance
2 weeks prior to Visit 1	• See Section 5.4, exclusion criterion 11.
12 hours prior to trial site admission at Visits 2 and 3 and until 6 hours after dosing of IMP	• Concomitant medications that are sensitive or moderately sensitive substrates of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP3A4 (*e.g., repaglinide, pioglitazone, losartan, linagliptin, saxagliptin, rivaroxaban)*. • Any statins.
12 hours prior to trial site admission at Visits 2 and 3 and until end of the EndoPAT assessment	• Any other concomitant medication. Subjects will be instructed to bring their medication, if any, with them on the day of the EndoPAT assessment to resume their medications right after the completion of assessment.

5.6 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.

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 (Visit 4). Any ongoing AEs will be followed-up as described in Section 9.6.

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.

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

5.6.1 Withdrawal from IMP

A subject will be withdrawn from IMP treatment if any of the following occurs:

- Sponsor decision to stop the trial or to stop the subject's participation in the trial; reasons may include medical, safety, or regulatory issues, or other reasons consistent with applicable laws, regulations, and GCP.
- Severe non-compliance to clinical trial protocol procedures, as judged by the investigator and/or Sponsor.
- It is the wish of the subject for any reason.
- The investigator judges it necessary due to medical reasons.
- Pregnancy.

A subject withdrawn from IMP at or after Visit 2 but before Visit 3, should not perform Visit 3 but return to the trial site for an end-of-trial visit (Visit 4).

5.6.2 Withdrawal from Trial

A subject will be withdrawn from the trial if any of the following occurs:

- It is the wish of the subject for any reason.
- Lost to follow-up.

5.7 Lost to Follow-up

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

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

- The site must attempt to contact the subject and reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule and ascertain whether the subject wishes to and/or should continue in the trial.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the subject (where possible, 3 telephone calls, and if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record.

- Should the subject continue to be unreachable, he/she will be considered to have withdrawn from the trial.

6 TRIAL DESIGN

6.1 Overall Trial Design

This is a randomized, double-blind, placebo-controlled, 2-way cross-over trial to evaluate the effect of C21 on endothelial dysfunction and safety in subjects with T2DM.

The subjects will have 4 visits at the trial site:

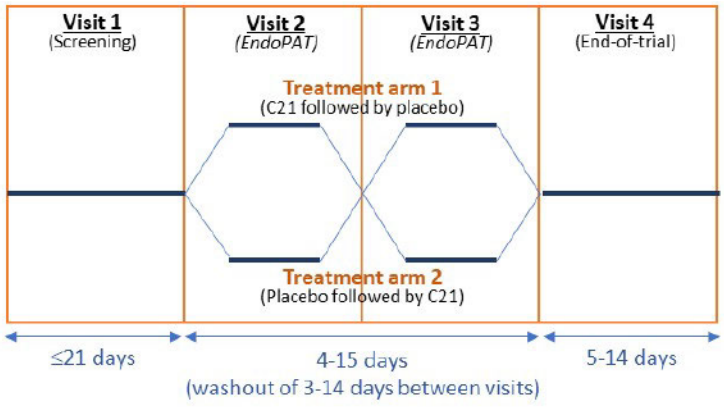
- Visit 1: Screening will take place within 3 weeks of the planned first IMP administration (Day -21 to Day -1) and will include the subject's signing of the informed consent and an eligibility check.
- Visits 2 and 3: Subjects will visit the trial site for single-dose, oral IMP administration (C21 or placebo according to randomization list), safety, tolerability and pharmacodynamic (EndoPAT) assessments. There will be a washout period of 3 to 14 days between Visits 2 and 3.
- Visit 4: A final end-of-trial visit will take place 5 to 14 days after Visit 3 or after early withdrawal from trial.

The duration of the trial for each subject will be 9 to 47 days, including an up to 3-week screening period.

6.2 Number of Subjects

The trial will include 10 to 14 randomised subjects, with 12 being the target number.

6.3 Trial Diagram



6.4 Trial Duration

Planned first subject screened: Quarter 2, 2023

Planned last subject randomised: Quarter 2, 2023

Planned last subject last visit: Quarter 3, 2023

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

6.5 Schedule of Events

Trial procedures will be performed according to the flow chart for trial procedures, Table 1, the trial treatment instructions in Section 7, and the trial assessment specifications in Section 8.

7 TRIAL TREATMENT

7.1 Investigational Medicinal Products

The IMP is the active (C21) and placebo treatments intended to be administered to a trial subject according to the clinical trial protocol (CTP) and the randomization list. The IMPs used in the trial are listed in Table 3.

[redacted]

Table 3. Investigational Medicinal Product Administered

IMP Name	Active (C21)	Placebo
Dose Formulation	Capsule	Capsule
[redacted]	[redacted]	[redacted]
Packaging and Labeling	IMP will be provided in a container. Each container will be labeled as required per country requirement.	IMP will be provided in a container. Each container will be labeled as required per country requirement.

7.2 Packaging, Labeling and Storage

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

The IMP will be manufactured, packed, labelled, and released to qualified person (QP) by Ardena Gent NV, Mariakerke, Belgium. The IMP will be packed in high-density polyethylene (HDPE) bottles with desiccant caps, containing 56 capsules per bottle.

Labeling of the IMP will be done in compliance with GMP Annex 13 (EU Guidelines to GMP 2009) and local regulatory requirements.

Only subjects enrolled in the trial may receive IMP, and only authorized site staff may supply or administer IMP. Prior to dispensing, all IMP must be stored at 15 to 25°C (59-77°F) in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

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

Further guidance and information on preparation, handling, storage, and accountability are provided in the IMP handling manual.

7.3 Treatment Assignment

Eligible subjects will be randomised to receive either C21 or placebo in a random order at Visit 2

[redacted]

7.4 Administration of Investigational Medicinal Products

All IMP will be administered under fasting conditions (see Section 5.5.1) and swallowed within a time span of 5 minutes with approximately 240 mL of tap water. The IMP will be administered 50±10 minutes before initiation of the EndoPAT assessment. The time point of dosing will be recorded in the eCRF.

IMP will be administered by the site staff and a trial staff member will oversee that the IMP is swallowed.

7.5 Compliance Check and Drug Accountability

When subjects are randomized at the site, they will receive the IMP doses directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The IMP container number and trial subject identification will be confirmed at the time of dosing by a member of the trial site staff other than the person dispensing the IMP.

A record of the quantity of IMP dispensed to and administered by each subject must be maintained and reconciled with IMP and compliance records.

After completed drug accountability, all unused and partly used IMP will be returned to sponsor or sponsor's designee for destruction or destructed at the site according to site procedures. All returned IMP will be reconciled at the end of the trial.

7.6 Overdose

An overdose is a dose in excess of the dose specified in this CTP.

In cases of accidental overdose of IMP, standard supportive measures will be adopted as required. No known antidotes and/or specified treatments are available in case of overdose with C21.

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.

7.7 Blinding of the Trial

This is a double-blind trial in which subjects, care providers, investigators, outcomes assessors, sponsor and any vendor staff are blinded to IMP allocation i.e., intervention assignment. The allocation of treatments will not be disclosed until clean file has been declared and the database has been locked.

7.8 Procedures for Unblinding

The sponsor or sponsor's designee will provide the trial site with one sealed randomisation code envelope per each randomised subject.

In case of an emergency, the investigator has the sole responsibility for determining if unblinding of a subjects' intervention assignment is warranted. Subject safety must always be the first consideration in making such a determination. If the investigator decides that unblinding is warranted, the investigator should make every effort to contact the sponsor prior to unblinding a subject's intervention assignment unless this could delay emergency treatment for the subject. If a subject's intervention assignment is unblinded, the sponsor must be notified within 24 hours of this occurrence. The date and reason for the unblinding must be recorded.

Sponsor pharmacovigilance staff or the pharmacovigilance vendor appointed by the sponsor may unblind the intervention assignment for any subject with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's intervention assignment, may be sent to the investigator in accordance with local regulations and/or sponsor policy. A blinding plan will describe any unblinded sponsor, or vendor staff during the conduct of the trial.

8 ASSESSMENTS

The trial assessments are described in the sections below and the timing of assessments are detailed in the flow chart for trial procedures (Table 1).

8.1 Demographics and Baseline Assessments

8.1.1 Informed Consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 15.2.

8.1.2 Eligibility Criteria

Eligibility criteria should be checked at screening (Visit 1) and verified before IMP administration at Visits 2 and 3. The criteria are specified in Sections 5.3 and 5.4.

8.1.3 Demographic Information

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

8.1.4 Height, Weight, and BMI

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

8.1.5 Medical/Surgical History and Concomitant Illness

Medical/surgical history will be obtained by subject interview to verify that the eligibility criteria are met. The medical/surgical history including date of first diagnosis of disease under trial and concomitant illnesses will be obtained by interviewing the subject or by checking his/her medical records.

8.2 Pharmacodynamic Assessments

8.2.1 EndoPAT

The endothelial vasodilator function will be assessed by EndoPAT at visits and time points specified in the flow chart for trial procedures (Table 1) according to the manufacturer's instructions.

The EndoPAT assessment will be conducted in a quiet, temperature-controlled room. Before initiating the assessment, the subject will be supine for at least 15 minutes. The subjects will be instructed to remain as still as possible during the assessment period. EndoPAT bio sensors will be placed on the subjects right and left index fingers. If this finger is unsuitable (*e.g.,* finger is cut or injured), a different finger may be used as long as the same finger is used on both hands.

The same finger should preferably be used at all assessments. A cuff will be placed on the nondominant upper arm for occlusion of the brachial artery.

Each recording will consist of a 5-minute baseline assessment, a 5-minute occlusion assessment, and 5-minute post-occlusion assessment. During post-occlusion period, blood flow is restored causing an endothelium-dependent vasodilation.

The EndoPAT software, provided with the device, will calculate the following outcomes using a computerised automated algorithm and recorded in the eCRF:

- Reactive Hyperemia Index (RHI) score: post-to-pre occlusion pulse amplitude tonometry (PAT) signal ratio in the occluded arm relative to the same ratio in the control arm, and corrected for baseline vascular tone. RHI is a measure of endothelial function.
- Augmentation index (AI): calculated from PAT pulses at the baseline period. AI is a measurement of vascular stiffness and reflects the structural nature and basal tonus of the vessel.

The time points of the initiation and completion of the EndoPAT assessment, the fingers assessed and any significant deviations from the instructions for use will be recorded in the eCRF.

8.3 Safety Assessments

8.3.1 Adverse Events

Adverse events (AEs) will be reported from signing of informed consent until end-of-trial participation (Visit 4).

At Visits 2 and 3, the subjects will be asked about AEs at admission to the trial site and just before discharge.

8.3.2 Pregnancy Test

Women of childbearing potential* will undergo pregnancy tests (urine dip-sticks) at all visits. If the urine pregancy test is positive, a blood pregnancy test will be performed to confirm positive pregnany.

*Women of non-childbearing potential are defined as pre-menopausal females who are sterilised (tubal ligation or permanent bilateral occlusion of fallopian tubes); or post-menopausal defined as 12 months of amenorrhea.

8.3.3 Physical Examination

A physical examination will be performed based upon the Investigator's judgement and may include the following:

- Head-eyes-ear-nose-throat
- Cardiovascular system
- Respiratory system
- Nervous system
- Gastrointestinal system
- Musculo-skeletal system

- Urogenital system
- Dermatology system
- Others, if required

As a minimum, assessments of cardiovascular system and respiratory system must be performed at Visits 1 and 4.

Symptom-driven physical examinations will be done upon admission and discharge from the trial site at Visit 2 and 3. 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.

8.3.4 Vital Signs

Systolic and diastolic blood pressure, pulse and body temperature will be measured in supine position after 10 minutes of rest at visits specified in the flow chart for trial procedures (Table 1). At Visits 1, 2 and 3, vital signs will be measured after IMP administration (applicable for Visits 2 and 3) and before initiating the EndoPAT assessment.

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.

8.3.5 Electrocardiogram

A 12-lead ECG will be recorded in supine position after 10 minutes of rest at visits specified in the flow chart for trial procedures (Table 1). At visits 2 and 3, ECGs will be recorded within 60 minutes prior to IMP dosing and again prior to initiating the EndoPAT assessment, 50±10 minutes after the IMP dosing. The ECGs will be reviewed and interpreted on-site by the investigator or designee.

Any ECG findings outside of normal ranges will be specified and documented as clinically significant or not clinically significant. The assessment and the time point of ECG collection will be recorded in the eCRF. Abnormal post-IMP administration findings assessed by the investigator as clinically significant will be reported as AEs.

8.3.6 Safety Laboratory Assessments

Blood samples for the analysis of clinical chemistry and haematology parameters will be collected through venepuncture and analysed by routine analytical methods.

The safety laboratory parameters are defined in Table 4 and will be assessed at visits specified in the flow chart for trial procedures (Table 1).

Any laboratory values outside of normal ranges will be specified and documented as 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 4. Safety laboratory parameters

Category	Parameter
Clinical chemistry	Alanine aminotransferase (ALT)
	Albumin
	Alkaline phosphatase (ALP)
	Aspartate aminotransferase (AST)
	Bilirubin (total and conjugated)
	Calcium
	Creatinine (eGFR included)
	C-reactive protein (CRP)
	Gamma glutamyl transferase (gGT)
	Glucose
	Potassium
	Sodium
	Urea
Haematology	Erythrocyte count
	Leukocyte count with differential count
	Haematocrit (B-EVF)
	Haemoglobin (Hb)
	Platelet count
FSH test (at the screening visit, postmenopausal females only)	Follicle stimulating hormone (FSH)

eGFR: Estimated glomerular filtration rate.

Any laboratory abnormality, judged by the investigator to be a clinically relevant worsening since Visit 1, should be reported as AEs if the laboratory abnormality required clinical intervention or further investigation, unless the laboratory abnormality is associated with an already reported event.

8.4 Other Assessments

8.4.1 Prior and Concomitant Medication

Prior medications taken will be obtained by subject interview in order to verify that the eligibility criteria are met.

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

9 ADVERSE EVENTS

9.1 Adverse Event Definitions

An adverse event (AE) is defined as any untoward medical occurrence in a subject administered the IMP and which may or may not have a causal relationship with this IMP. An AE can therefore be any unfavorable and unintended sign (e.g. a significant abnormal laboratory finding, symptom, or disease temporally associated with the use of the IMP, whether or not considered related to the IMP.

An adverse drug reaction (ADR) is any untoward and unintended response to an IMP assessed as related to any dose administered. Response means that the AE is assigned a causality assessment of "Related" to IMP.

An AE is considered "unexpected" if the nature, severity, or outcome is not consistant with the reference safety information in the current edition of the IB.

A Treatment Emergent Adverse Event (TEAE) is any sign, event or symptom that emerges during treatment having been absent pre-treatment, or worsens relative to the pre-treatment state.

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it was more severe.)
- Requires in-patient hospitalisation or prolongation of existing hospitalisation*.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or 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 subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

*Exemptions may be made when hospitalization is due to:

- Routine treatment or monitoring of the investigated indication, not associated with any deterioration in the condition.
- Treatment that was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under investigation and did not worsen.
- Admission to a hospital or other institution for general care, not associated with any deterioration in the condition.

9.2 Other Reportable Information

Certain information although not consistent with an SAE must be recorded, reported, and followed up in the same way as an SAE. This includes:

- Any pregnancy that occurs during trial participation must be reported and administration of IMP must be terminated immediately. A pregnancy must be reported immediately to sponsor or designee. The pregnancy must be followed up to determine outcome including premature termination and status of mother and infant. The outcome must be reported even if there are no abnormal findings. Pregnancy complications and elective terminations for medical reasons must be reported as AEs or SAEs, as applicable. Spontaneous abortion must be reported as an SAE. In addition, the investigator must attempt to collect pregnancy information on any female partners of male trial participants who become pregnant while the male participant is enrolled in the trial. Pregnancy information must be reported to sponsor as described above.
- Lactation exposure to and IMP with or without an AE.
- Overdose of an IMP with or without an AE.
- Inadvertent or accidental exposure to an IMP with or without an AE.

9.3 Adverse Event Assessment Definitions

9.3.1 Severity

The investigator should assess the severity of all AEs according to the following definitions:

- Mild: awareness of symptoms, signs, illness, or event that is easily tolerated.
- Moderate: discomfort sufficient to cause interference with usual activity.
- Severe: incapacitating with inability to work or undertake further normal activities.

Note the distinction between seriousness and severity: The term severe is used to describe the intensity of the event and a severe event is not necessarily serious. The seriousness criteria serve as a guide for defining regulatory reporting obligations.

9.3.2 Relationship to IMP

Assessment of causality is based on the following considerations: associative connections (time and/or place), pharmacological explanations, previous knowledge of the drug, presence of characteristic clinical or pathological phenomena, exclusion of other causes, and/or absence of alternative explanations.

The investigator will assess the relationship to trial treatment for all AEs and SAEs. The relationship will be characterized using the following causality ratings:

- Related
- Not related
- Not applicable

Causality assessment instructions are as follows:

Related: There is a reasonable possibility that the AE was caused by the drug. There is a reasonable time relationship to drug 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 patient did not receive any treatment with IMP.

9.3.3 Outcome

The investigator will be asked to record the most appropriate outcome of the following categories:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved/ongoing
- Recovered/resolved with sequelae
- Fatal
- Unknown

9.4 Recording of Adverse Events

All events meeting the definition of an AE must be reported in the period from the subject has signed the informed consent form until the end-of-trial participation (Visit 4).

At each visit the subject will be asked about AEs in an objective manner, e.g.: "Have you experienced any problems since the last visit?". They will also be asked if they have been hospitalized, had any accidents, usen any new medications, or changed concomitant medication regiments (both prescription and over the counter medication).

Only medically qualified personnel are allowed to assess AEs.

AEs must be reported on the AE page of the eCRF. The diagnosis should be recorded, if available. If no diagnosis is available each sign and symptom should be recorded as individual AEs.

Information to be collected includes type of event, time of onset, investigator's assessment of severity and relationship to trial treatment, time of resolution of the event, seriousness, as well as any required treatment or evaluations, and outcome. The AEs resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. For follow-up of AEs please refer to Section 9.6.

The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened for the trial and which does not deteriorate should not be reported as an AE. However, if the condition deteriorates/ worsens at any time during the subject's participation in the trial, it should be recorded as an AE.

Abnormal laboratory values shall be recorded as AEs if assessed as clinically significant by the investigator. In that case, the severity will be assessed as per above. Laboratory abnormalities that are associated with an already reported medical condition will not be reported as separate AEs but will be used for the assessment of the associated medical condition (e.g., increased neutrophil count in case of a reported infection or increased glucose in a patient with diabetes).

Changes in the severity of an AE should be recorded to allow an assessment of the duration of the event at each level of intensity to be performed. An AE characterized as intermittent requires documentation of the onset and duration of each episode.

9.5 Reporting of Serious Adverse Events

Any AE which meets the definition of a SAEs must be reported within 24 hours of obtaining knowledge of the event. The information to be reported will include assessment of severity, causal relationship to IMP or trial procedures, action taken, outcome, and a narrative description of the course of the event.

The sponsor is responsible for notifying the relevant Health Authorities in compliance with applicable legislation. All suspected unexpected serious adverse reactions (SUSARs) are subject to expedited reporting to Health Authorities.

The investigator is responsible for notifying Ethics Committees (ECs) directly as per EC requirements.

The trial specific SAE form must be used when reporting to the sponsor.

Additional information may be subsequently provided.

The SAE form and all other relevant documents supporting the reported SAE (e.g. diagnostic procedures, hospital records, autopsy reports) must be faxed or scanned/emailed to the sponsor or sponsor's designee using the following e-mail address Vicore@primevigilance.com.

9.6 Follow-up on Adverse Events

All AEs should be followed until they have reached a final outcome (Recovered/resolved; Recovering/resolving; Not recovered/not resolved/ongoing; Recovered/resolved with sequelae; Fatal, Unknown) or the subject's participation in the trial ends, whichever comes first.

SAEs and severe, non-serious AEs considered related to IMP still ongoing after the trial end should be followed on a regular basis according to the investigator's clinical judgment until a final outcome has been established. The outcome category "Not recovered/not resolved/ongoing" may used as a final outcome in situations where the severity grade is no longer subject to change.

10 CHANGES TO TRIAL CONDUCT

10.1 Protocol Amendments

Before implementation of substantial changes (as defined in EU Clinical Trials Directive (2001/EC/20)) unless considered an Urgent Safety Measure, approval/favourable opinion must be obtained from the appropriate regulatory authority(ies) and IEC(s).

10.2 Premature Termination of the Trial

In case of premature termination of the trial, health authorities and IECs will be notified in writing, including the reason for premature termination.

Conditions that may warrant premature termination of the trial include, but are not limited to the following:

- The discovery of an unexpected and significant or unacceptable risk to the subjects enrolled in the trial.
- A decision of the sponsor to discontinue development of the drug.

10.3 Premature Termination of a Trial Site

The sponsor can decide to prematurely terminate a site. Conditions that may warrant termination include, but are not limited to:

- Insufficient adherence to protocol requirements.
- Failure to enrol subjects at an acceptable rate.

11 DATA HANDLING AND RECORD KEEPING

11.1 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).

11.2 Data from Clinical Trial Site

Data from clinical trial site will be entered in an eCRF. The eCRF will be a 21 CFR part 11 compliant, password protected and validated system.

The investigator will sign relevant eCRF sections by use of an electronic signature. Only the investigator (i.e., medically qualified personnel) can sign data on medical assessments. Any corrections made by the investigator or authorised staff to the eCRF after original entry will be documented in an audit trail. The person making the change and the date, time and reason for the change will be identified in the audit trail. Changes to the data already approved/signed by an investigator will require re-signature by the investigator. The investigator (principal investigator or sub-investigator) will sign all patient data in the eCRF by an electronic signature.

Subject data will be recorded pseudonymized and the subjects will be identified only by a screening number and a randomisation number.

The monitor will check the eCRF for accuracy and completion and perform source data verification. Data entered in the eCRF will be verified against source data.

11.3 Source Data

Source documents containing source data provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.

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.

The monitor will perform ongoing source data verification to confirm that data recorded in the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the trial is being conducted in accordance with the currently approved protocol and any other trial agreements, ICH GCP, and all applicable regulatory requirements.

The level of source data verification will be described in the monitoring plan.

11.4 Coding of Data

Medical history and AEs will be coded using the lastest version of Medical Dictionary for Regulatory Activities (MedDRA).

Concomitant medication will be coded using the latest version of World Health Organisation (WHO) Drug Reference List.

11.5 Subject Confidentiality

The confidentiality of the subject data and subject records shall be protected, respecting the privacy and confidentiality rules in accordance with applicable regulatory requirements.

12 RETENTION OF DOCUMENTS

The monitor will instruct the investigator to maintain source documents and the signed informed consent form for each subject.

Furthermore, the monitor will instruct the investigator to archive essential documents for the duration defined in the ICH GCP R2 or for 15 years, whichever comes first.

The sponsor will notify the investigator when retention of the trial-related records is no longer required.

13 STATISTICAL METHODS

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.

13.1 General

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).

Baseline (apart from EndoPAT assessments) 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.

13.2 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.

13.3 Analysis Data Sets

The Safety Set will consist of all subjects who have been randomised and received at least one dose of IMP. This analysis set will be used for safety assessments.

The PD Analysis Set (PDAS) will consist of all subjects who have been randomised and received at least one dose of IMP, provided evaluable PD variables from either Visit 2 or 3, and who have no AE or protocol deviations judged to affect the PD analysis. Individual PD values may be excluded from the analysis as specified in the SAP.

The PD Ratio Analysis Set (PDRAS) will consist of all subjects who have completed the trial up until Visit 3, provided evaluable PD variables from both Visits 2 and 3, and who have no AEs or protocol deviations judged to affect the PD analysis. Individual PD values may be excluded from the analysis as specified in the SAP.

13.4 Subject Disposition

13.4.1 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.

13.4.2 Medical/surgical history

Medical history will be presented by system-organ-class (SOC) and preferred term (PT). All data will be listed by subject.

13.4.3 Prior/concomitant medication

Prior/concomitant medications will be presented by ATC level 4 and 5. All data will be listed by subject.

13.4.4 IMP administration

All administered individual IMP administrations will be listed by subject.

13.5 Pharmacodynamic Analysis

The EndoPAT software, provided with the device, will calculate the following outcome using a computerised, automated algorithm:

- Reactive Hyperemia Index (RHI) score: post-to-pre occlusion pulse amplitude tonometry (PAT) signal ratio in the occluded arm relative to the same ratio in the control arm, and corrected for baseline vascular tone. RHI is a measure of endothelial function.
- Augmentation index (AI): calculated from PAT pulses at the baseline period. AI is a measurement of vascular stiffness.

13.5.1 Analysis of Primary Endpoint

The percentage change in the primary endpoint (RHI) will be compared between treatments using an analysis of variance model (ANOVA) adjusting for subject (random), period and treatment. The adjusted mean difference will be presented together with 90% confidence interval and associated two-sided p-value.

All data will be listed by subject.

13.5.2 Analysis of Secondary/Exploratory Endpoint

The secondary endpoint (AI) will be compared between treatments using similar ANOVA model as for the primary endpoint.

13.5.3 Statistical/Analytical Issues

All tests will be two-sided at a 10% significance level. There will be no adjustment for multiple tests.

13.5.3.1 Missing Data

There will be no imputation of pharmacodynamic or safety data.

13.5.3.2 Examination of Subgroups

Important demographic and baseline value-defined subgroups will be examined and the results presented, *e.g.*, comparison of effects by age, sex and baseline disease severity (T2DM). A detailed description will be provided in the SAP.

13.6 Safety Analysis

13.6.1 Adverse Events

An overview of all treatment-emerging AEs including severity, relationship to IMP, SAEs and AEs leading to withdrawals of treatment and withdrawal from trial or death will be presented. The incidence of treatment-emerging AEs and SAEs will be summarised by system organ class (according to MedDRA) and preferred term (according to MedDRA) by treatment.

All treatment-emergent AEs will be listed by subject and include the verbatim term entered by the investigator.

13.6.2 Pregnancy

All pregnancy test results will be listed by subject.

13.6.3 Physical examination

Abnormal findings on the physical examination will be summarised by visit.

All physical examination data will be listed by subject.

13.6.4 Vital Signs

Vital signs data (e.g., blood pressure and body temperature) will be summarised by visit.

All vital signs data will be listed by subject.

13.6.5 Electrocardiogram

All ECGs will be categorised as ”normal”, ”abnormal, not clinically significant”, or ”abnormal, clinically significant” (as judged by the investigator). The assessment will be summarised by visit and time point relative to IMP administration.

All ECG data will be listed by subject.

13.6.6 Laboratory Safety Assessments

All clinical laboratory values outside of normal ranges will be categorised as ”abnormal, not clinically significant”, or ”abnormal, clinically significant” as judged by the investigator.

All clinical laboratory data (*i.e.,* values outside of normal ranges and categorisation) will be listed by subject.

14 GOOD CLINICAL PRACTICE

This trial will be carried out in compliance with the protocol, current ICH GCP, standard operating procedures, the EU Clinical Trials Directive (2001/20/EC), and applicable local regulatory requirements.

The investigator agrees, when signing this protocol, to adhere to the instructions and procedures described in it, to the principles of the Declaration of Helsinki, current ICH GCP, and applicable regulatory requirements.

In compliance with ICH GCP R2 requirements, sponsor will identify those processes and data that are critical to ensure human subject protection and the reliability of trial results and furthermore, identify, evaluate, control, communicate and review risks to those.

15 ETHICS

15.1 Independent Ethics Committees / Health Authorities

Before implementing this trial, the protocol, the proposed subject information and subject consent form, and other documents as required, will be reviewed by properly constituted Independent Ethics Committees (IECs) and by the national regulatory authorities.

A signed and dated statement that the protocol and subject information and subject consent form have been approved by the IECs and regulatory authorities will be obtained before trial initiation.

For each individual IEC the name and occupation of the chairman and the members of the IEC will be collected as well as a statement that the IEC works in accordance with ICH GCP.

IECs will receive updates on trial progress according to local regulations.

15.2 Informed Consent

The subject's signed and dated informed consent to participate in the trial will be obtained prior to any trial related procedure being carried out.

Before any trial related procedure the investigator will explain to the potential subject the aims, methods, reasonably expected benefits and potential hazards of the trial and any discomfort participation in the trial may entail. Subjects will be informed that participation in the trial is voluntary and that the subject may withdraw from the trial at any time and for any reason. Subjects will be informed that if they choose not to participate, this will not affect the care the subject will receive for the treatment of his or her disease. Finally, subjects will be informed that their records may be accessed by health authorities and authorized sponsor staff without violating the confidentiality of the subject, to the extent permitted by the applicable laws or regulations.

All subjects will be given opportunity to ask questions and will be given sufficient time to consider before consenting. The subjects may choose to be accompanied, *e.g.* by a family member, during the information process. After having consented, a copy of the informed consent form will be given to the subject.

15.3 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.

15.4 Subject Privacy and Data Protection

The informed consent 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), these pseudonymised data will not identify any subjects 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 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 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 pseudonymized, 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 pseudonymized 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 any subcontractors used in the trial are data processors.

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

16 AUDITS AND INSPECTIONS

A representative of the sponsor may visit the trial site(s) at any time during the trial or after completion of the trial to conduct an audit of the trial. These audits will require access to all trial records, including source documents, for inspection and comparison with the eCRFs. The

investigator and other trial personnel will be responsible for being present and available for consultation during routinely scheduled site audit visits conducted by the sponsor's representative.

Similar auditing procedures (inspections) may also be conducted by agents of regulatory health authorities, either as part of a national GCP compliance program or to review the results of this trial in support of a regulatory submission. The investigator should notify the sponsor's representative or sponsor immediately, if he/she has been contacted by a regulatory agency concerning an upcoming inspection.

17 MONITORING

Monitoring will be conducted by a sponsor representative. Before trial initiation the monitor will review the protocol and the eCRF with the investigator(s) and their trial personnel.

As described in the monitoring plan, the monitor will periodically visit the trial site to check the completeness of subject records, the accuracy of entries in eCRFs, the adherence to the protocol and to GCP, the progress of enrolment and the handling and accounting of IMP. Key trial personnel must be available to assist the monitor during these visits.

The investigator must give the monitor direct access to source data/documents (e.g. relevant hospital or medical records) to confirm their consistency with the entries in eCRFs. No information in these records about the identity of the subjects must leave the trial site.

18 REPORTING OF RESULTS

18.1 Clinical Trial Report

Data will be reported in an integrated clinical trial report in compliance with the requirements of the current version of ICH E3: Structure and Content of Clinical Study Report. The signatory investigator will review and sign the integrated clinical trial report.

18.2 Use of Information

All unpublished information relating to this trial and/or to the trial drug is considered confidential by the sponsor and shall remain the sole property of the sponsor.

The investigator must accept that the sponsor may use the information from this clinical trial in connection with the development of the IMP, and therefore, may disclose it as required to other investigators, to government licensing authorities, to regulatory agencies of other governments, stock exchange market, and commercial partners.

18.3 Publication of Results

The sponsor is committed to publishing the trial results, whether positive or negative, in a peer-reviewed journal.

The criteria for authorship as set out by the Committee of Medical Journal Editors (www.icmje.org) will be applied (Wager et al., 2003; Graf et al., 2009).

The contributorship model will be applied and contributors who do not meet the criteria for authorship will be listed in an acknowledgments section with descriptions of the role of each contributor in order to ensure indexation in the National Library of Medicine.

Publications are subject to the following conditions:

- Data are the property of the sponsor and cannot be published without prior authorization from the sponsor.
- Publications should be drafted with protection of individual privacy, intellectual property and contract rights in mind, and also conform to legislation and current national practices in patent and other laws.
- The primary publication (*i.e.* the results from all centers) should be published before, or in parallel with, any secondary publications.
- Publications shall not disclose any sponsor confidential information or property.

19 INSURANCE AND LIABILITY

The sponsor has subscribed to an insurance policy covering, in its terms and provision, its legal liability for injuries caused to participating subjects and arising out of trial procedures performed in accordance this protocol, in accordance with applicable law and with ICH GCP R2.

20 REFERENCES

Graf, C., Battista, W. P., Bridges, D., Bruce-Winkler, V., Conaty, J. M., Ellison, J. M., Field, E. A., Gurr, J. A., Marx, M. E., Patel, M., Sanes-Miller, C., Yarker, Y. E. International Society for Medical Publication Professional. 2009 Good publication practice for community company Sponsored medical research: the GPP2 guidelines. BMJ 339:b4330.

ICH Harmonized Tripartite Guideline E3. 1995 Structure and Content of Clinical Study Reports. Step 5.

ICH Harmonized Tripartite Guideline E6 (R2). 1997 Step 5: Guideline for Good Clinical Practice. Adopted by CPMP Dec 2016.

Matrougui, K., Loufrani, L., Heymes, C., Levy, B. I., and Henrion, D. 1999 Activation of AT(2) receptors by endogenous angiotensin II is involved in flow-induced dilation in rat resistance arteries. Hypertension 34:659–665.

Steckelings, U. M., Widdop, R. E., Sturrock, E. D., Lubbe, L., Hussain, T., Kaschina, E., Unger, T., Hallberg, A., Carey, R. M., and Sumners, C. 2022 The angiotensin AT2 receptor: from a binding site to a novel therapeutic target. Pharmacol. Rev. 74:1051–1135.

Wager, E., Field, E. A., Grossman, L. 2003 Good publication practices for pharmaceutical companies: why we need another set of guidelines. Curr. Med. Res. Opin. 19(3):147-8.

21 ABBREVIATIONS

Abbreviation	Explanation
ADR	Adverse Drug Reaction
AE	Adverse Event
AI	Augmentation Index
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
AST	Aspartate Aminotransferase
AT1R	Angiotensin II Type 1 Receptor
AT2R	Angiotensin II Type 2 Receptor
ATC	Anatomical Therapeutic Chemical
ATRAG	Angiotensin II Type 2 Receptor Agonist
AUC	Area Under the Curve
AV	Atrioventricular
BID	Twice daily (lat. *bis in die*)
BMI	Body Mass Index
BCRP	Breast cancer resistance protein
BUN	Blood Urea Nitrogen
C21	Compound 21
Cmax	Maximum plasma concentration
COVID-19	Coronavirus Disease 2019
CNS	Central Nervous System
CRF	Case Report Form
CRP	C-reactive protein
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 Interactions
eCRF	electronic Case Report Form
ECG	Electrocardiography
EoT	End of Treatment
EMA	European Medicines Agency
eNOS	Endothelial nitric oxide synthase
ERA	Endothelin receptor antagonist
EU	European Union
FSH	Follicle-stimulating Hormone
FU	Follow-up
GCP	Good Clinical Practice
gGT	Gamma Glutamyl Transferase
GLP-1	Glucagon-Like Peptide-1
GMP	Good Manufacturing Practise
Hb	Haemoglobin
HBsAg	Hepatitis B virus s-Antigen
HCG	Human Chorionic Gonadotropin
HCVAb	Hepatitis C virus antibodies

HIV	Human Immunodeficiency Virus
HDL	High-density Lipoprotein
hs-CRP	high-sensitivity C-Reactive Protein
IB	Investigator's Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IPF	Idiopathic Pulmonary Fibrosis
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LD	Lactate dehydrogenase
LDL	Low-density Lipoprotein
LFT	Liver Function Test
MDR1	Multi-Drug Resistance Protein 1
MedDRA	Medical Dictionary for Regulatory Activities
NO	Nitric oxide
NOAEL	No Observed Adverse Effect Level
PD	Pharmacodynamic
PK	Pharmacokinetic
$PPAR_{\gamma}$	Peroxisome Proliferator-Activated Receptor-gamma
OAT3	Organic Anion Transporter 3
OATP1B1	Organic Anion Transporting Polypeptide 1B
QTcF	QT interval with Fridericia's correction
RAS	Renin-Angiotensin System
RHI	Reactive Hyperemia Index
RP	Raynaud's Phenomenon
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SLC	Solute Carrier
SSc	Systemic Sclerosis
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2DM	Type 2 Diabetes Mellitus
TSH	Thyroid Stimulating Hormone
WHO	World Health Organization
WHODrug	WHO Drug Dictionary
WOCBP	Women of Childbearing Potential