Study title

A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED PHASE I TRIAL TO INVESTIGATE SAFETY, TOLERABILITYAND PHARMACOKINETICS OF SINGLE ASCENDING TOPICAL DOSES OF GZ21T IN HEALTHY VOLUNTEERS

NCT Number

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Clinical Trial Protocol

v2.0; 20NOV2024

Clinical Trial Protocol

EU Trial No.	2024-512893-86-00
Investigational medicinal product	GZ21T
Trial code	A24-0001
Protocol version and date	Final v2.0; 20NOV2024

A DOUBLE-BLIND, RANDOMISED, PLACEBO-CONTROLLED PHASE I TRIAL TO INVESTIGATE SAFETY, TOLERABILITY AND PHARMACOKINETICS OF SINGLE ASCENDING TOPICAL DOSES OF GZ21T IN HEALTHY VOLUNTEERS

Phase	I
Test product and dose	GZ21T cream, [redacted] Part A: Dose: [redacted] *(applied to [redacted] body surface area [BSA])* Part B: Dose [redacted] *(applied to [redacted])*
Comparator product and dose	Placebo cream, 0 mg/g Dose: [redacted] *(applied to [redacted])*
Sponsor signatory	[redacted]
Principal Investigator	Mohammad Alimohammadi, MD, PhD CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden
Clinical trial conduct and management	Clinical Trial Consultants AB (CTC) CTC Akademiska, Uppsala University Hospital, Entrance 85, 2nd level SE-751 85 Uppsala, Sweden and/or CTC Oscar, Dag Hammarskjölds väg 10C SE-752 37 Uppsala, Sweden

The following amendments have been made to the first submitted version of this Clinical Trial Protocol (version 1.0)

Type of change	Date	Summary of changes	Revised protocol version
Substantial amendment	20NOV2024	An additonal part (Part B) has been added to include 1 cohort (3 particpants), and up to 2 optional cohorts with 3 participants in each cohort, in order to evaluate the optimum amount of cream to be applied to a given body area (dose/area).	Version 1.0

1 TRIAL SYNOPSIS

Trial title A double-blind, randomised, placebo-controlled Phase I trial to investigate safety, tolerability, and pharmacokinetics of single ascending topical doses of GZ21T in healthy volunteers.	
Trial code A24-0001	**EU Trial No.** 2024-512893-86-00
Planned trial period Q2 2024 to Q1 2025	**Phase of development** I
Principal Investigator Mohammad Alimohammadi, MD, PhD CTC Clinical Trial Consultants AB Dag Hammarskjölds väg 10B SE-752 37 Uppsala, Sweden	
Trial background The Sponsor, Ankh Life Sciences Limited, is developing GZ21T as a topical treatment of actinic keratosis. GZ21T is a fixed combination of isovanillin, curcumin, and harmine that has been shown to have anti-tumour activity, partially due to the activation of the kinase ataxia-telangiectasia mutated (ATM) and the adenosine monophosphate (AMP)-dependent protein kinase (AMPKα) T172, in addition to enhanced autophagy. *In vitro* studies on actinic keratosis cells have demonstrated that GZ21T induces 2 complementary cell-killing processes that converge on the mitochondrion to cause caspase-dependent and -independent cell death.	
Trial design and trial population This is a Phase I trial divided in 2 parts. Part A is double-blind, randomised, placebo-controlled and designed to evaluate safety, tolerability, pharmacokinetics (PK), after topical administration of single ascending doses of GZ21T in healthy volunteers. Part B is open-label and designed to evaluate the optimum amount of cream applied to a given body area (dose/area) of single doses of GZ21T in healthy volunteers.	
Objectives **Part A** <u>Primary objective</u> • To evaluate the safety and tolerability of GZ21T after single topical dose applications. <u>Secondary objective</u> • To investigate the potential systemic exposure and PK properties of GZ21T after single topical dose applications. **Part B** <u>Primary objective</u> • To investigate the optimum amount of GZ21T cream applied to a given area (dose/area) after single topical dose applications.	

Endpoints

Part A

Primary endpoint

- Frequency, severity and intensity of adverse events (AEs).
- Local tolerability reactions:
  - Erythema, swelling, pruritus, burning, blistering and urticaria, discoloration and dryness (Investigator's assessment 0-3 none/mild/moderate/severe).
- Clinically significant changes in vital signs, electrocardiograms (ECGs), safety laboratory parameters, and physical examination findings.

Secondary endpoints

- Plasma concentrations of GZ21T after single dose applications.
- PK parameters after a single dose application (to be calculated if data permits): area under the plasma concentration curve from time 0 to infinity (AUC_{inf}), AUC from time 0 to the last measurable concentration (AUC_{last}), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($T_{½}$).

Additional PK parameters may be determined if data permits and if deemed appropriate.

Part B

Primary endpoint

- Cream absorption after single dose applications.

Number of participants planned

Part A: Approximately, 64 potential participants will be screened to achieve 32 randomised participants (4 cohorts, 8 participants per cohort randomised in a 6:2 ratio).

Part B: Approximately 18 potential particpants will be screened to achieve up to 9 included particpants, 3 subjects per cohort.

Diagnosis and eligibility criteria

Healthy male or female participants aged 18 to 70 years (inclusive) with a body mass index (BMI) ≥ 18.5 and ≤ 30.0 kg/m^2 at the time of the screening visit who are willing and able to give informed consent for participation in the trial. Female volunteers of childbearing potential must agree to use a highly effective method of contraception during the trial period to prevent pregnancy. Male volunteers must agree to use adequate contraception during the trial period to prevent pregnancy of a partner of childbearing potential.

Methodology

Part A

Participants will receive a single topical application of GZ21T or placebo in 2 sequential cohorts followed by 2 cohorts starting in parallel with planned doses as follows:

- Cohort 1: [redacted] GZ21T or placebo will be applied to [redacted] of the skin, corresponding to approximately [redacted] body surface area (BSA) and [redacted] g cream.
- Cohort 2: [redacted] GZ21T or placebo will be applied to [redacted] of the skin, corresponding to approximately [redacted] and [redacted] g cream.
- Cohort 3: [redacted] GZ21T or placebo will be applied to [redacted] of the skin, corresponding to approximately [redacted] % BSA and [redacted] g cream.
- Cohort 4: [redacted] GZ21T or placebo will be applied to the face, corresponding to approximately [redacted] and [redacted] g cream.

Participants will come for 3 visits to the research clinic for screening, treatment, and follow-up.

Screening (Visit 1) will take place between Day -28 and Day -1. Eligible participants will be admitted to the research clinic between Day 1 and Day 2 (Visit 2) for randomisation, investigational medicinal product (IMP) administration and pre- and post-dose safety and PK assessments.

In each cohort, a single dose of the IMP will be topically applied to the selected area. The treated area will be marked with a medical skin waterproof marker. During that time, participants should remain still without touching the treated area. Before treatment on Day 1, participants must have abstained from using moisturising ointments, creams, or emollients for 24 hours on the area to be treated.

Sentinel dosing will be applied. The first 2 participants in each cohort will receive either GZ21T or placebo as randomised. After the sentinel participants, at least 24 hours must pass before dosing of the remaining participants to give sufficient time for the observation of any reactions.

All participants will be carefully monitored by clinical staff during and after IMP application and will remain at the research clinic for at least 24 hours after treatment (Day 2) for safety assessments, including safety laboratory testing, 12-lead ECG, vital signs, local tolerability, physical examination and AEs, and PK assessments. Participants will return to the research clinic on Day 7±1 day (Visit 3) for final safety and PK assessments.

Before initiating a new cohort, all participants in the previous cohort(s) must have been dosed and observed for at least 24 hours for safety and tolerability and to obtain blood samples for PK analysis. The PK data, up to and including 24 hours data, for all treated participants must have been reviewed by the internal safety review committee (iSRC) before initiating a new cohort. Once safety, tolerability, and PK data of the last participant in each cohort has been collected, there must be at least 1 week between dose escalations to allow for a thorough evaluation of data.

Part B

Participants will receive a single topical application of GZ21T.

- Cohort 1: [redacted] mg/cm^2 GZ21T will be applied to [redacted] cm^2 of the skin, corresponding to approximately [redacted]% BSA and [redacted] g cream. Up to 2 optional cohorts may be added. The doses in the optional cohorts may be higher or lower but will not exceed the doses in Part A.

Participants will come for 3 visits to the research clinic for screening, treatment, and follow-up, and will have 1 remote telephone visit. Screening (Visit 1) will take place between Day -28 and Day -1. Eligible participants will visit the research clinic on Day 1 (Visit 2) for IMP administration and cream absorption evaluation and local tolerability assessments.

A single dose of the IMP will be topically applied to the selected area. The treated area will be marked with a medical skin waterproof marker. During that time, participants should remain still without touching the treated area. Before treatment on Day 1, participants must have abstained from using moisturising ointments, creams, or emollients for 24 hours on the area to be treated.

All participants will be carefully monitored by clinical staff during and after IMP application and will remain at the research clinic for 2 hours after treatment for local tolerability and AE evaluation. On Day 2 (Visit 3), 24 hours post-dose, participants will visit the research clinic for follow-up of local tolerability and AEs. A remote telephone call will be performed on Day 7 ±1 day (Visit 4) to follow-up on local tolerability and AEs.

Investigational Medicinal Product, dosage and mode of administration

The IMPs will be provided as GZ21T cream, 15 mg/g (1.5%), tubes of each 7 gram, and placebo for GZ21T cream, 0 mg/g, tubes of each 7 grams.

Doses for Part A:

- Cohort 1: [redacted] g GZ21T or placebo will be applied to [redacted] cm^2 of the skin, corresponding to approximately [redacted]% BSA.
- Cohort 2: [redacted] g GZ21T or placebo will be applied to [redacted] cm^2 of the skin, corresponding to approximately [redacted]% BSA.
- Cohort 3: [redacted] g GZ21T or placebo will be applied to [redacted] cm^2 of the skin, corresponding to approximately [redacted]% BSA.
- Cohort 4: [redacted] g GZ21T or placebo will be applied to the face, corresponding to approximately [redacted]% BSA.

Doses for Part B:

- Cohort 1: [redacted] g GZ21T will be applied to [redacted] cm^2 of the skin. The doses for the optional cohorts (cohorts 2 and 3) will be decided based on the results from the previous cohorts. The doses will not exceed the doses given in Part A.

Duration of treatment

Single topical dose application.

Duration of each participant's involvement in the trial

In both parts, each participant is expected to participate in the trial for a maximum of 35 days including an up to 28-days screening period.

Safety assessments

- AEs
- Local tolerability
- Clinical laboratory parameters
- Vital signs (blood pressure, pulse)
- 12-lead ECG
- Physical examinations

Pharmacokinetic assessments

Part A: Blood sampling for subsequent bioanalysis of GZ21T plasma concentrations and calculation of PK parameters.

Ethical Considerations and Benefit-risk Analysis

While keeping the identified risk factors at a minimum level in order to not expose the participants 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 participants.

Statistical methods No formal sample size calculation has been performed. The proposed sample size is considered sufficient to provide adequate information for the trial endpoints. Data will be summarised using descriptive statistics as appropriate. All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).
Trial reporting After completion of the trial, an International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E3 guideline-compliant clinical trial report (CTR) will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
ADHD	Attention deficit hyperactivity disorder
ADL	Activities of daily living
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AMP	Adenosine monophosphate
AMPKα	AMP-activated protein kinase
APTT	Activated partial thromboplastin clotting time
AR	Adverse reaction
AST	Aspartate aminotransferase
ATC	Anatomical therapeutic chemical
ATM	Ataxia-telangiectasia mutated
AUC	Area under the plasma concentration *vs.* time curve
AUC_{inf}	AUC from 0 to infinity
AUC_{last}	AUC from 0 to the time of the last measurable plasma concentration
B-EVF	Blood erythrocyte volume fraction
BID	Two times daily (*Latin bis in die*)
BMI	Body mass index
BSA	Body surface area
bw	Body weight
C_{max}	Maximum observed concentration
CRO	Contract research organisation
CTIS	Clinical Trials Information System (EU portal)
CTP	Clinical trial protocol
CTR	Clinical trial report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
DBL	Database lock
DMP	Data management plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram

Abbreviation	Explanation
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
FAS	Full analysis set
FDA	United States Food and Drug Administration
FIH	First-in-human
FSH	Follicle-stimulating hormone
GCP	Good clinical practice
GDP	Good distribution practice
GDPR	General data protection regulation
GMP	Good manufacturing practice
Hb	Haemoglobin
hCG	Human chorionic gonadotropin
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICTRP	International clinical trials registry platform
IEC	Independent ethics committee
IMP	Investigational medicinal product
ISF	Investigator site file
iSRC	Internal safety review committee
IUD	Intra-uterine device
IUS	Intra-uterine system
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical dictionary for regulatory activities
NIH	National Institutes of Health
NOAEL	No observed adverse effect level
NSAID	Non-steroidal anti-inflammatory drug

Abbreviation	Explanation
PD	Protocol deviation
PII	Personally identifiable information
PK	Pharmacokinetic(s)
PK(INR)	Prothrombin complex international normalised ratio
PKAS	PK analysis set
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
RBM	Risk-based monitoring
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SAS	Statistical analysis software
SD	Standard deviation
SDV	Source data verification
SMP	Safety management plan
SOC	System organ class
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial master file
T_{max}	Time of occurrence of C_{max}
$T_{1/2}$	Terminal elimination half-life
WHO	World Health Organization
WOCBP	Women of childbearing potential

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contact

The Principal Investigator 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 11.3.1.14.

In the case of a medical emergency, the Investigator may, during office hours, contact the Medical Monitor (Table 4.1-1).

Table 4.1-1 Medical emergencies contact

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

5 INVESTIGATOR AND TRIAL ADMINISTRATIVE STRUCTURE

Sponsor

Ankh Life Sciences

[redacted]

Sponsor's Medical Representative and Chief Operating Officer

[redacted]

Clinical conduct

Clinical Trial Consultants AB

CTC Akademiska,
Uppsala University Hospital,
Entrance 85, 2nd level
SE-751 85 Uppsala, Sweden

and/or

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

Principal Investigator

Mohammad Alimohammadi, MD, PhD

[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]

Laboratory (safety)

Clinical Microbiology
Uppsala University Hospital
Dag Hammarskjölds väg 38
SE-752 37 Uppsala, Sweden

	Clinical Chemistry and Pharmacology Uppsala University Hospital Entrance 61, 2nd level SE-751 85 Uppsala, Sweden
Laboratory (bioanalysis)	Scantox Sweden Astra Zeneca BioVentureHub, Pepparedsleden 1, SE-431 53 Mölndal, Sweden
Investigational medicinal product (IMP) manufacturing, packaging, labelling and qualified person (QP) release	[redacted]
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

Actinic keratosis is a disease characterised by lesions of epidermal keratinocyte dysplasia that result from chronic sun exposure and may progress to invasive squamous cell carcinoma [1]. Actinic keratosis is caused by repeated, prolonged exposure to ultraviolet light, and typically affects Caucasians. Current standard-of-care treatments of actinic keratosis include cryo-ablation of lesions, surgery, and/or topical treatment with medicated creams including 5- fluorouracil, imiquimod, and diclofenac.

The Sponsor, Ankh Life Sciences Limited, is developing GZ21T as a topical treatment of actinic keratosis. GZ21T is a fixed combination of isovanillin (77%), curcumin (10%), and harmine (13%) that has been shown to have anti-tumour activity, due to enhanced autophagy and due to the activation of the kinase ataxia-telangiectasia mutated (ATM) and the adenosine monophosphate (AMP)- activated protein kinase (AMPKα) T172. ATM is a serine/threonine kinase that acts upstream of p53 and controls a deoxyribonucleic acid (DNA) damage response pathway critical to resolving single-stranded DNA breaks. *In vitro* studies on actinic keratosis cells have shown that GZ21T induces 2 complementary cell-killing processes that converge on the mitochondrion to cause caspase-dependent and -independent actinic keratosis cell death.

Product characteristics

Genzada Pharmaceutical's oral drug compound, GZ17-6.02, is a fixed-dose combination of 3 synthetically synthesised active pharmaceutical ingredients. Isovanillin, harmine, and curcumin are combined in a fixed ratio of 77%, 13%, and 10 % by weight, respectively, to create GZ17-6.02. The IMP is a topical formulation, GZ21T, that has been developed to study the effect of locally applied GZ17-6.02 for the treatment of actinic keratosis. The drug product is presented as a creme formulation containing [redacted] (w/w) of the active drug compound GZ17-6.02, *i.e.*, [redacted]

Mechanism of action

GZ17-6.02/GZ21T act on actinic keratosis cells through multiple convergent mechanisms. *In vitro* studies have demonstrated the mechanisms include such processes as decreased cellular survival signalling via inhibition of the AMPK/Mammalian target of rapamycin signalling cascade, decreased epidermal growth factor receptor signalling, and effects on additional canonical signalling pathways. For details, refer to the Investigator's Brochure (IB).

6.1.1 Summary of non-clinical data

In pre-clinical studies, GZ17-6.02 has demonstrated anti-proliferative activity against a variety of human solid tumours, multiple myeloma, and lymphoma cell lines *in vitro*, and efficacy in human pancreatic cancer and head and neck squamous cell carcinoma using orthotopic or subcutaneous athymic mouse models. There is also pre-clinical data supporting the development of a topical formulation to treat pre-cancerous lesions, such as actinic keratosis [2].

[redacted]

6.1.2 Clinical experience

In a first-in-human (FIH) Phase 1 clinical trial, GZ17-6.02 was well tolerated when orally administered to oncology patients with solid tumours and lymphoma at a dose of [redacted]375mg twice a day (daily dosage equivalent 750 mg).

[redacted]

Thus, there is clinical experience regarding GZ17-6.02 at higher doses and longer treatment duration than for the proposed topical trial.

[redacted] The PK analysis showed that all PK samples were below the detection limit.

Preliminary and blinded data showed that GZ21T has been generally well tolerated, without clinically significant fingings regarding AEs, ECG, safety laboratory assessments, physical examinations and local tolerability findings. [redacted]

6.2 Trial rationale

GZ21T cream is intended to be studied as a potential treatment for patients with actinic keratoses and other dermatologic conditions which may be amendable to the study treatment.

This is a Phase I trial in healthy participants divided in 2 parts. The aim for Part A is to collect information about safety, tolerability, and pharmacokinetics (PK) following a single topical administration of the GZ21T cream. The objective of Part B is to determine the appropriate amount of cream for use in the multiple ascending dose trial and for daily application. The trial is intended to provide important information to support the design of further studies in patients with actinic keratosis.

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 well-being are of utmost importance. The trial involves the first administration of GZ21T as a topical formulation to humans, and there are no previous data on the effects of this topical formulation and with this route of administration in humans. It is therefore difficult to make predictions about possible adverse reactions (ARs).

Although previously performed toxicology studies and a Phase 1 clinical trial in humans using the GZ17-6.02 oral formulation have shown that the compound safety profile was acceptable and manageable for the patient population that was studied, there is still a need for attention to risk mitigation.

Since there are no data available at this stage on reproductive toxicology, participants included in the trial must be of non-childbearing potential or, if of childbearing potential, must practise abstinence or must agree to use a highly effective method of contraception.

The selection of starting dose and dose escalation steps for Part A represents a careful approach to administering the drug topically for the first time in humans (see Section 8.3.1). Sentinel dosing will apply to the first 2 participants in each cohort. An internal safety review committee (iSRC) will monitor the emerging safety, tolerability, and PK data throughout the trial and will recommend the next dose level prior to any dose escalation. After the collection of the last 24 hours of data, there will be at least 1 week between dose escalation.

Participants in Part A will remain in the trial site for 24 hours after IMP administration and will be closely monitored by medical staff. Visits at the site may be prolonged if the Investigator finds it medically warranted for safety reasons.

Overdosing is not likely to occur since all IMP will be applied by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures should be adopted as required. For further information, refer to Section 11.3.1.19.

Each participant will be provided with a participation card with information about their participation in a trial, refer to Section 14.4.

The Principal Investigator at the trial site will ascertain that adequate facilities and procedures are available to handle emergencies should they occur during the trial. The medical staff at CTC have extensive experience from early Phase trials, and there are adequate procedures in place to handle unexpected and expected ARs in the trial participants.

Aside from the risks related to GZ21T, 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 trials have not revealed any safety issues that would outweigh the expected benefits of the trial.

While keeping the risk mentioned above factors at a minimum level in order to not expose the participants taking part 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 participants.

More detailed information about the known and expected benefits and risks and reasonably expected ARs are found in the current version of the GZ21T IB (version 1.0).

7 TRIAL OBJECTIVES AND ENDPOINTS

The trial objectives and endpoints are summarised in Table 7.1-1 (Part A) and Table 7.1-2 (Part B).

7.1 Trial objectives and endpoints

Table 7.1-1 Trial objectives and endpoints (Part A)

Objectives	Endpoints	Assessments	Analysis
Primary			
To evaluate the safety and tolerability of GZ21T after single topical dose applications.	Frequency, severity and intensity of AEs.	AE reporting and questioning (Section 11.3.1).	Section 17.6.1.
	Local tolerability reactions: Erythema, swelling, pruritus, burning, blistering and urticaria, discoloration and dryness (Investigator's assessment 0-3 none/mild/moderate/severe).	Local tolerability reactions (Section 11.3.2).	Section 17.6.2.
	Clinically significant changes in vital signs, electrocardiograms (ECGs), safety laboratory measurements (haematology, clinical chemistry, coagulation) and physical examination findings.	Blood pressure and pulse (Section 11.3.3).	Section 17.6.3.
		12-lead ECG (Section 11.3.4).	Section 17.6.4.
		Blood sampling for haematology, clinical chemistry and coagulation (Section 11.3.5).	Section 17.6.5.
		Physical examinations (Section 11.3.6).	Section 17.6.6.
Secondary			
To investigate the potential systemic exposure and pharmacokinetic (PK) properties of GZ21T after single topical dose applications.	Plasma concentrations of GZ21T after single dose applications.	PK sampling and analysis (Section 11.4.1).	Section 17.7.1.
	PK parameters after a single dose application (to be calculated if data permits): area under the plasma concentration curve from time 0 to infinity (AUC_{inf}), AUC from time 0 to the last measurable concentration (AUC_{last}), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), terminal elimination half-life ($T_{½}$).		

Table 7.1-2 Trial objective and endpoint (Part B)

Objectives	Endpoints	Assessments	Analysis
Primary			
To investigate the optimum amount of cream applied to a given area (dose/area) after single topical dose applications.	Cream absorption after single dose applications.	Evaluation of cream absorption (Section 11.3.7).	Section 17.6.7

8 TRIAL DESIGN

8.1 Overall trial design and schedule of events

The trial is a Phase I trial divided into 2 parts. Part A is double-blind, placebo-controlled, and designed to evaluate the safety, tolerability, and PK after topical administration of single ascending doses of GZ21T in healthy male and female volunteers. Part B is open-label and designed to evaluate the optimum amount of cream applied to a given body area (dose/area) of single doses of GZ21T in healthy volunteers.

In Part A, participants will receive a single topical application of GZ21T or placebo in 2 sequential cohorts (cohorts 1 and 2) followed by 2 cohorts starting in parallel (cohorts 3 and 4) with planned doses as follows:

[redacted]

In Part B, participants in cohort 1 will receive a single topical application of GZ21T. Based on the extent of the cream's absorption, up to 2 additional cohorts can be explored (optional cohorts 2 and 3). The maximum administered dose will not exceed 25.5 mg/cm^2.

The trial design and the proposed dose levels are shown in Figure 8.1-1. The rationale for the proposed starting dose and the planned dose escalation is detailed in Section 8.3.

[redacted]

Part A

[redacted]

Part B

[redacted]

In Part A, participants will come for 3 visits to the research clinic for screening, treatment, and follow-up. Screening (Visit 1) will take place between Day -28 and Day -1. Eligible participants will be admitted to the research clinic between Day 1 and Day 2 (Visit 2) for randomisation, IMP administration and pre- and post-dose safety and PK assessments.

In each cohort, a single dose of the IMP will be topically applied to the selected area. The treated area will be marked with a medical skin waterproof marker. During that time, participants should remain still without touching the treated area. Before treatment on Day 1, participants must have abstained from using moisturising ointments, creams, or emollients for 24 hours on the area to be treated.

Sentinel dosing will be applied. The first 2 participants in each cohort will receive either GZ21T or a placebo as randomised. After the sentinel participants, at least 24 hours must pass before dosing of the remaining participants to give sufficient time for the observation of any reactions.

All participants will be carefully monitored by clinical staff during and after IMP application and will remain at the research clinic for at least 24 hours after treatment (Day 2) for safety assessments, including safety laboratory testing, 12-lead electrocardiogram (ECG), vital signs, local tolerability and AEs, and PK assessments. Participants will return to the research clinic on Day 7±1 day (Visit 3) for final safety and PK assessments.

Before initiating a new cohort, all participants in the previous cohort(s) must have been dosed and observed for at least 24 hours for safety and tolerability and to obtain blood samples for PK analysis. The PK data, up to and including 24 hours data, for all treated participants must have been reviewed by the iSRC before initiating a new cohort. Once safety, tolerability, and PK data of the last participant in each cohort has been collected, there must be at least 1 week between dose escalations to allow for a thorough evaluation of data.

Each participant is expected to participate in Part A of the trial for a maximum of 35 days including an up to 28-day screening period.

The overall schedule of events for Part A of the trial is shown in Table 8.1-1, and the detailed schedule of events for Visit 2 is shown in Table 8.1-2. Trial assessments are described in Section 11.

In Part B, Participants will come for 3 visits to the research clinic for screening, treatment, and follow-up, and will have 1 remote telephone visit. Screening (Visit 1) will take place between Day -28 and Day -1. Eligible participants will visit the research clinic on Day 1 (Visit 2) for IMP administration cream absorption evaluation and local tolerability assessments.

A single dose of the IMP will be topically applied to the selected area. The treated area will be marked with a medical skin waterproof marker. During that time, participants should remain still without touching the treated area. Before treatment on Day 1, participants must have abstained from using moisturising ointments, creams, or emollients for 24 hours on the area to be treated.

All participants will be carefully monitored by clinical staff during and after IMP application and will remain at the research clinic for 2 hours after treatment for local tolerability and AE evaluation. On Day 2 (Visit 3), approximately 24 hours post-dose, participants will visit the research clinic for follow-up of local tolerability and AEs. A remote telephone call will be performed on Day 7 ±1 day (Visit 4) to follow-up on local tolerability and AEs.

Each participant is expected to participate in Part B of the trial for a maximum of 35 days, including an up to 28-day screening period.

The schedule of events for Part B of the trial is shown in Table 8.1-3 and the detailed schedule for Visit 2 and Visit 3 is shown in Table 8.1-4. Trial assessments are described in Section 11.

Table 8.1-1 Overall schedule of events (Part A)

		Screening	Inpatient stay		Follow-up/End-of-trial
		Visit 1	Visit 2[1]		Visit 3[2]
Assessment↓/Day→	**CTP section**	**Day -28 to Day -1**	**Day 1**	**Day 2**	**Day 7 ±1 day**
Informed consent	11.2.1	X			
Eligibility criteria	9.4/9.5	X	X[3]		
Demographics	11.2.3	X			
Weight/height/BMI/BSA[4]	11.2.4	X			
Medical/surgical history	11.2.5	X			
HIV, hepatitis B and C	11.2.6	X			
Physical examination	11.3.6	X	X	X[5]	X[5]
Pregnancy test[6]	11.2.7	X	X		
Urine drug screen	11.2.8	X	X		
Alcohol test	11.2.9	X	X		
Safety laboratory profile[7]	11.3.5	X	X	X	X
12-lead ECG	11.3.4	X	X	X	X
Vital signs[8]	11.3.3	X	X	X	X
Local tolerability[9]	11.3.2		X	X	X
Randomisation	9.9		X		
IMP administration	10.5		X		
PK blood sampling	11.4.1		X	X	X
Meals[10]	-		----------X----------		
Baseline symptoms[11]	11.2.10	X			
Adverse events[12]	11.3.1		----------X----------		
Prior and concomitant medications	11.2.11	----------X----------			

BMI=body mass index, BSA=body surface area, ECG=electrocardiogram, HIV=human immunodeficiency virus, IMP=investigational medicinal product, PK=pharmacokinetic,

1. Detailed schedule for Visit 2 is shown in Table 8.1-2.
2. Or after early withdrawal.
3. Confirmation of eligibility criteria, prior to IMP administration.

4. BSA will be calculated from the weight and height.
5. Symptom-driven physical examination.
6. Women of childbearing potential (WOCBP) only. At screening: plasma/serum test. At other visits: urine test.
7. Clinical chemistry, haematology, and coagulation.
8. Blood pressure, and pulse.
9. Dryness and discoloration will be assessed pre-dose, and 24 hours and 7 days post-dose.
10. Meals Day 1: breakfast (optional), lunch, snack (optional), dinner, and evening snack will be served during the day. Meals Day 2: breakfast only.
11. Baseline symptoms will be recorded up until the administration of IMP on Day 1.
12. AEs will be recorded from the first administration of IMP.

Table 8.1-2 Detailed schedule of events for Visit 2 (Part A)

Day→	**Day 1**											**Day 2**
Assessment↓/Time→	**Pre-dose**	**0**	**30 min**	**1 h**	**2 h**	**3 h**	**4 h**	**6 h**	**8 h**	**10 h**	**12 h**	**24 h**
Eligibility criteria	X											
Physical examination	X											X[1]
Urine drug-screen	X											
Alcohol test	X											
Pregnancy test (WOCBP)	X											
Safety laboratory profile	X											X
12-lead ECG	X				X				X			X
Vital signs	X				X				X			X
Local tolerability[2]	X				X				X			X
Randomisation	X											
IMP administration		X										
PK blood sampling	X		X	X	X	X	X	X	X	X	X	X
Meals[3]	——————————————————————————————————X——————————————————————————————————											
Baseline symptoms	X											
Adverse events		——————————————————————————————————X——————————————————————————————————										
Prior and concomitant medications	——————————————————————————————————X——————————————————————————————————											

ECG=electrocardiogram, IMP=investigational medicinal product, PK=pharmacokinetic, WOCBP=women of childbearing potential

1. Symptom-driven physical examination.
2. Dryness and discoloration will be assessed pre-dose and 24 hours post-dose.
3. Meals Day 1: breakfast (optional), lunch, snack, dinner, and evening snack. Lunch, snack, dinner, and evening snack (optional) will be served during the day. Meals Day 2: breakfast only.

Table 8.1-3 Overall schedule of events (Part B)

		Screening	Treatment visit	Follow-up	Follow-up (telephone call) /End-of-trial
		Visit 1	**Visit 2[1]**	**Visit 3[1]**	**Visit 4[2]**
Assessment↓/Day→	**CTP section**	**Day -28 to Day -1**	**Day 1**	**Day 2**	**Day 7 ±1 day**
Informed consent	11.2.1	X			
Eligibility criteria	9.4/9.5	X	X[3]		
Demographics	11.2.3	X			
Weight/height/BMI/BSA[4]	11.2.4	X			
Medical/surgical history	11.2.5	X			
HIV, hepatitis B and C	11.2.6	X			
Physical examination	11.3.6	X			
Pregnancy test[5]	11.2.7	X	X		
Urine drug screen	11.2.8	X	X		
Alcohol test	11.2.9	X	X		
Safety laboratory profile[6]	11.3.5	X			
12-lead ECG	11.3.4	X			
Vital signs[7]	11.3.3	X			
Local tolerability[8]	11.3.2		X	X	
IMP administration	10.5		X		
Cream absorption evaluation	11.3.7		X		
Baseline symptoms[9]	11.2.10	----------X----------			
Adverse events[10]	11.3.1		----------X----------		
Prior and concomitant medications	11.2.11	----------X----------			

BMI=body mass index, BSA=body surface area, ECG=electrocardiogram, HIV=human immunodeficiency virus, IMP=investigational medicinal product

1. Detailed schedule for Visit 2 and Visit 3 is shown in Table 8.1-4.
2. Or after early withdrawal.
3. Confirmation of eligibility criteria, prior to IMP administration.
4. BSA will be calculated from the weight and height.
5. WOCBP only. At screening: plasma/serum test. At other visits: urine test.

6. Clinical chemistry, haematology, and coagulation.
7. Blood pressure, and pulse.
8. Local tolerability assessments will be done 2 hours post-dose. Dryness and discoloration will be assessed pre-dose, and 24 hours post-dose.
9. Baseline symptoms will be recorded up until the administration of IMP on Day 1.
10. AEs will be recorded from the first administration of IMP.

Table 8.1-4 Detailed schedule of events for Visit 2 and Visit 3, Part B

Day→	**Day 1**						**Day 2**
Assessment↓/Time→	**Pre-dose**	**0**	**15 min**	**30 min**	**1 h**	**2 h**	**24 h**
Eligibility criteria	X						
Urine drug-screen	X						
Alcohol test	X						
Pregnancy test (WOCBP)	X						
Local tolerability	X					X[0]	X[2]
IMP administration		X					
Cream absorption evaluation (photography)			X	X	X	X	
Cream absorption evaluation (visual inspection by trial staff)				X	X	X[0]	
Baseline symptoms	X						
Adverse events		——————————————————————X——————————————————————					
Prior and concomitant medications	——————————————————————————————————X——————————————————————————————————						

WPBCP=women of childbearing potential

1. Particpants will be discharged from the research clinic after the local tolerability and cream absorption assessments at 2 hours.
2. Dryness and discoloration will be assessed pre-dose and 24 hours post-dose.

8.2 Rationale for trial design

The European Medicines Agency (EMA) guideline EMEA/CHMP/SWP/28367/07 Rev. 1 on strategies to identify and mitigate risks for FIH and early clinical trials [3] has been considered when designing this trial.

In Part A, the design of the trial is based on the aim to study the safety, tolerability, and PK of selected doses of GZ21T in a limited number of healthy male and female volunteers. The trial involves careful monitoring of the participant's well-being and will provide important safety and PK data to support the design of further trials, both in healthy volunteers and in patients. The length of the trial and time points for PK blood sampling were selected based on data obtained from previous non-clinical and clinical trials.

A placebo control will be used to establish the frequency and magnitude of changes in primary and secondary endpoints that may occur in the absence of active treatment, in compliance with the relevant International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [4-6].

Randomisation will be used to minimise bias in the assignment of participants to treatment groups and to increase the likelihood that known and unknown participant attributes (*e.g.*, demographic and baseline characteristics) are evenly balanced.

Blinded treatment will be used to reduce potential bias during data collection and the evaluation of endpoints.

In Part B, the design of the trial is based on the aim to study the optimal amount of cream suitable for daily use to be applied in a limited number of healthy male and female volunteers. In order to achieve the objectives of this part of the trial, a placebo group is not required.

8.3 Selection of starting dose and rationale for planned dose escalation

8.3.1 Selection of starting dose

The drug compound G17-6.02 has previously been given orally to approximately 40 patients with solid tumours and lymphoma, at dose levels up to [redacted] mg two times daily (BID). In that study, 375 mg G17-6.02 BID was determined to be maximum tolerated dose and the recommended dose for future studies.

The developed cream formulation of G17-6.07, named GZ21T to be used in this study, contains [redacted] of the drug compound (G17-6.07). ***In Part A***, the start dose of [redacted] ***In Part B***, the dose given in cohort 1 will be approximately 50% of the start dose in Part A and will not exceed the doses given in Part A in subsequent cohorts (optional cohorts 2 and 3).

The minipig skin is considered a most relevant model for human skin in terms of toxicity and absorption. In the 28-day dermal toxicity study in minipigs, the GZ21T [redacted] % cream was applied once or twice daily to skin areas corresponding to [redacted] % of BSA and in local concentrations of [redacted] mg (drug compound)/cm^2. The total doses administered per kg body weight (bw) were [redacted] mg/kg. No local tolerability issues or systemic effects were recorded in this study.

8.3.2 Rationale for dose choices

Refer to Section 8.3.1.

8.3.3 Maximum dose

The maximum dose used in the trial will not exceed [redacted] GZ21T cream applied to [redacted] BSA.

8.3.4 Stopping criteria for dose escalation (Part A)

Dose escalation and stopping criteria are shown in Table 8.3-1.

The Principal Investigator and the iSRC (see Section 8.3.5) will follow the recommendations and grading system of the common terminology criteria for adverse events (CTCAE) v5.0 [7] (see Section 11.3.1) as well as the recommendations published by *Sibille et al. (2010)* [8], which constitute an FIH adaptation of the grading systems previously proposed in the CTCAE v5.0, the World Health Organisation (WHO) recommendations for grading acute and subacute toxicity in cancer treatment [9], the National Institutes of Health (NIH) grading of severity of adult and paediatric AEs [10], and the US Food and Drug Administration (FDA) toxicity grading scale for healthy adult and adolescent volunteers included in preventive vaccine clinical trials [11]. The intensity grade and frequency of AEs will be considered (refer to Section 11.3.1).

Emerging trends or safety signals, which are not necessarily covered by the stopping criteria in Table 8.3-1 may warrant the scheduling of *ad hoc* iSRC meetings, after which the iSRC will make recommendations to the Sponsor on whether to stop dosing individual participants or certain cohorts, or whether to terminate the trial altogether.

No systemic toxicity was observed when GZ21T at doses up to [redacted] bw were applied topically to minipigs for 28 days. Furthermore, there was very low systemic exposure to GZ21T in the study. It is therefore difficult to establish stopping rules based on GZ21T exposure which have been reflected in the stopping rules defined in Table 8.3-1. A thorough evaluation with respect to safety criteria will be performed.

Table 8.3-1 Dose escalation and stopping criteria

No.	Stopping criterion	Action taken
At the individual and cohort level		
1	If no participant has an serious adverse event (SAE) considered at least possibly related to the IMP administration, *i.e.*, a serious adverse reaction (SAR).	Escalate to the next higher dose level.
2	If 1 participant on active treatment has an SAE assessed as at least possibly related to IMP administration.	1. Stop dosing the participant with a potential SAR. Await the dosing of additional participants until the iSRC's recommendation and the Sponsor's decision on continuation. 2. Unblind the participant with potential SAR. Only voting members of the iSRC will be unblinded. 3. Evaluation by the iSRC. The iSRC makes a recommendation to the Sponsor. 4. The Sponsor decides how to proceed. The participant received active treatment, *i.e.,* met the stop criterion: stop further dosing at this dose level for all participants. The Sponsor decides if the trial will be terminated or if dosing will resume at a lower dose level.
3	If 2 participants on active treatment in the same cohort have severe, non-serious AEs assessed at least possibly related to the IMP administration (independent of whether the AEs are within the same system organ class [SOC]).	1. Stop dosing the participants with severe, non-serious potential ARs. 2. Unblind the participants with potential ARs. Only voting members of the iSRC will be unblinded. 3. Evaluation by the iSRC. The iSRC makes a recommendation to the Sponsor. 4. The Sponsor decides how to proceed. Both participants received active treatment, *i.e.*, met the stop criterion: stop further dosing at this dose level for all participants. The Sponsor decides if the trial will be terminated or if dosing should resume at a lower dose level.
Final dosing stop and termination of trial		
4	If 2 participants on active treatment have SAEs assessed as at least possibly related to IMP administration.	1. Stop dosing the participants with potential SARs. Await dosing additional participants until the iSRC's recommendation and the Sponsor's decision on continuation. 2. Unblind the participants with potential SARs. Only voting members of the iSRC will be unblinded. 3. Evaluation by the iSRC. The iSRC makes a recommendation to the Sponsor. 4. The Sponsor decides how to proceed. Both participants received active treatment, *i.e.,* met the stop criterion: Termination of trial. One (1) participant received active treatment, *i.e.*, met stop criterion: See stop criterion no. 2.

8.3.5 Internal Safety Review Committee (Part A)

The voting members of the iSRC will consist of the Principal Investigator, the CTC Medical Monitor and the Sponsor's Medical Representative. In addition, the trial clinical research manager, the trial pharmacokineticist and additional Sponsor representatives will be invited as appropriate and function as non-voting members. Other internal or external experts may be invited and consulted by the iSRC as appropriate.

Before initiating a new dose cohort, all participants in the previous cohort must have been treated and all available safety, tolerability, and plasma concentration data up until and including 24 hours after dosing must have been evaluated by the iSRC. After collection of the last available 24-hour data, there will be at least 1 week between dose escalations. In case of dropouts, available data for the dropouts will be included in the iSRC evaluation. If the GZ21T drug is considered safe and tolerable, the recommended next dose level, based on the predicted exposure, will be provided to the Sponsor in writing. Based on the emerging safety and plasma PK data, the amount of required data to be reviewed after each completed cohort may be adjusted. Likewise, the number of dose levels and planned sampling times may also be adjusted. These adjustments will be documented in non-substantial modifications to the CTP. Details regarding the timing of iSRC reviews and the data to be reviewed will be provided in a separate iSRC charter.

Sentinel dosing will be applied. The first 2 participants in each cohort will receive either GZ21T or placebo as randomised. After the sentinel participants, at least 24 hours must pass before dosing of the remaining participants to give sufficient time for the observation of any reactions.

An adaptive dosing strategy will be applied to allow for a flexible and safe dose escalation. The planned dose escalation is outlined in Section 8.3.2. The actual doses administered in each cohort will be guided by the iSRC recommendations based on the available safety, tolerability, and PK data, as described above. Every dose step is thus adjustable and the recommendation to the Sponsor may be to continue with a higher or lower dose than the planned dose, to repeat the same dose level, to continue with an intermediate dose level, or to stop dosing.

The recommendations of the iSRC will be made in consensus between the iSRC members and documented as appropriate. In case of disagreement between the voting members, the most conservative approach will be taken. Repeating a dose level will not be allowed if any of the dose escalation-stopping criteria described in Section 8.3.4 have been met.

The treatment code for individual participants may be requested to be broken by the iSRC voting members during the assessment process (partial unblinding) in accordance with the stopping criteria described in Section 8.3.4 to enable their decision on continued dosing of further cohorts or to stop the dose escalation. The medical staff will still be blinded for the treatments (active drug or placebo) to be administered in the subsequent cohorts in order to minimise bias. If unblinding was considered necessary, the iSRC meeting may consist of a closed part and an open part.

9 TRIAL POPULATION

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

9.1 Recruitment

Participants 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 authorised 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 participants even if they were not subsequently included in the trial. This information is necessary to verify that participants were selected without bias. The reason for screening failure should be stated for all participants screened but not included. The reason for withdrawal should be stated for all participants who 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 participant in connection to the informed consent process at the screening visit (Visit 1). The screening number will allow the identification of participants irrespective of their possible eligibility for the trial.

Eligible participants will be assigned a 3-digit randomisation number prior to the first IMP administration. For details, refer to Section 9.9.

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

9.3 Number of participants

Part A: Approximately, 64 potential participants will be screened to achieve 32 randomised participants (4 cohorts, 8 participants per cohort randomised in a 6:2 ratio).

Part B: Approximately 18 potential particpants will be screened to achieve up to 9 included particpants.

For the replacement of participants who discontinue the trial, see Section 9.8.3.

9.4 Inclusion criteria

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

1. Willing and able to give written informed consent for participation in the trial.
2. Healthy male or female participant aged 18 to 70 years, inclusive.
3. Body Mass Index (BMI) $\geq$ 18.5 and $\leq$ 30.0 kg/m^2 at the time of the screening visit.
4. **WOCBP** must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the participant) or must agree to use a highly effective method of contraception with a failure rate of <1 % to prevent pregnancy from at least 2 weeks prior to the administration of IMP to 4 weeks after the last administration of IMP.

In addition, any male partner of a female participant must, unless he is sterile (*e.g.*, has undergone vasectomy), agree to use a condom from the first administration of IMP until 4 weeks after the last administration of IMP.

The following are considered highly effective methods of contraception:

- 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),
- intra-uterine device [IUD]or intra-uterine hormone-releasing system [IUS]).

WOCBP must refrain from donating eggs from the first IMP administration until 3 months after the last IMP administration.

Women of non-childbearing potential are 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).

Male participants, if not sterile (*e.g.*, vasectomised), must be willing to use a condom or practice sexual abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the participant) to prevent pregnancy and drug exposure of a partner and 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 participant who is of childbearing potential must use contraceptive methods with a failure rate of < 1% to prevent pregnancy (see above) 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

Participants 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 participant at risk because of participation in the trial or influence the results or the participant's ability to participate in the trial.
2. Any clinically significant illness, medical/surgical procedure, or trauma within 4 weeks of the administration of IMP.
3. Any clinically significant abnormalities regarding physical examination, vital signs, 12- lead ECG, and laboratory values at the time of the screening visit, as judged by the Investigator.
4. Malignancy within the past 5 years, including removal of basal cell carcinoma.
5. Any planned major surgery within the duration of the trial.
6. Any skin condition including tattoos that may limit the evaluation of *e.g.*, local tolerability as judged by the Investigator.
7. History of chronic urticaria, known history of urticaria triggered by specific factors or currently experiencing an episode of urticaria within the past 3 months.
8. History of psoriasis, atopic eczema and similar conditions, as judged by the Investigator.
9. Prescence of body hair or tattoos on the intended application areas, which in the opinion of the Investigator could interfere with local tolerability assessments.

10. Females who are pregnant, currently breastfeeding, or intend to become pregnant during the course of the trial.
11. Any positive result at the screening visit for serum hepatitis B surface antigen, hepatitis B and C antibodies and/or human immunodeficiency virus (HIV).
12. After 10 minutes of 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
13. Prolonged QTcF (>450 ms), cardiac arrhythmias or any clinically significant abnormalities in the resting ECG at the screening visit, as judged by the Investigator.
14. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs or food with a similar chemical structure or class to GZ21T.
15. Regular use of any prescribed or non-prescribed medications, including antacids, analgesics, herbal remedies, 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.
16. Unwillingness to abstain from the use of topical treatment (including but not limited to corticosteroids, calcineurin inhibitors, vitamin D analogues, and retinoids) at the application site within 1 week prior to Day 1 and from the use of moisturising ointment cream, emollients, oils (including shower oil) or sunscreen within 24 hours prior to Day 1 until 1 week after IMP administration.
17. Planned treatment or treatment with another investigational drug within 3 months prior to Day 1. Participants who consented and screened but were not dosed in previous clinical trials are not to be excluded.
18. Current smokers or users of nicotine products. Irregular use of nicotine (*e.g.*, smoking, snuffing, chewing tobacco) less than 3 times per week is allowed before screening visit.
19. Positive screening result for drugs of abuse or alcohol at the screening visit or on admission to the trial site prior to the administration of the IMP. (Positive results that are expected given the participant's medical history and prescribed medications can be disregarded as judged by the Investigator.)
20. History of alcohol abuse or excessive intake of alcohol, history or presence of drug abuse including anabolic steroids, as judged by the Investigator
21. Plasma donation within approximately 1 month of screening or blood donation (or corresponding blood loss) during the last 3 months prior to screening, at the discretion of the Investigator.
22. The Investigator considers the participant unlikely to comply with trial procedures, restrictions and requirements.

9.6 Restrictions during the trial

Participants 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: Participants are expected to use contraceptive methods in accordance with inclusion criterion #4 or practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the participant) during the clinical trial.
2. Alcohol: Consumption of alcohol is not allowed within 48 hours prior to admittance to the trial site including the end-of-trial visit.
3. 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.
4. Nicotine: Smoking or use of nicotine products (*e.g.*, snuff, chewing tobacco) are not allowed from the screening visit to the end-of-trial visit.
5. Exercise: The participants will be asked to abstain from strenuous exercise, defined as greater than 70% of the maximal pulse rate for ≥ 1 hour, for 48 hours prior to each trial visit.
6. Blood donation: The participants must not donate blood or plasma from the screening visit until 3 months after the final medical examination at the end-of-trial visit.
7. Participation in other clinical trials: The participants are not allowed to participate in any other interventional clinical trial from the screening visit until the end-of-trial visit.
8. Leaving the trial clinic: Participants are not allowed to leave the trial site during trial visits unless authorised by the trial personnel.
9. Moisturising creams: Participants must have abstained from using moisturising ointments, creams, or emollients (including shower oil and sunscreen) on the area to be treated for 24 hours prior to treatment on Day 1, until the end-of-trial visit.
10. Showering: Participants must refrain from washing the treated area or taking a shower from the IMP administration until the 24-hour assessment on Day 2 (Part A).
11. Sun protection: Protection from the sun is required from the IMP administration until the end-of-trial visit (Day 7). Participants are recommended to:
    - Stay in the shade, especially during the sun's peak hours of 10 AM to 4 PM.
    - Wear clothing that covers the treated skin area.
    - Abstain from the use of tanning beds.

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 administration of IMP is not allowed, as judged by the Investigator. This includes antacids, analgesics and herbal remedies.

Topical treatments are not allowed at the application site within 1 week prior to IMP administration.

The use of any prescribed or non-prescribed medication from the administration of IMP until the end-of-the trial visit will not be allowed except as detailed below.

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 participant, withdrawal should be considered.
- Nasal decongestants without cortisone, antihistamine, or anticholinergics for a maximum of 10 days.
- Non-steroidal anti-inflammatory drugs (NSAIDs) intermittent use for dysmenorrhoea and dysmenorrhoea-associated symptoms, as judged by the Investigator.
- Contraceptives, as defined in inclusion criterion #4.
- Vitamins and minerals.

As detailed in exclusion criterion #18, a positive drug-screen will exclude a participant from participation in the trial. However, positive results that are expected given the participant's medical history and prescribed medications (*e.g.*, opioid analgesics or attention deficit hyperactivity disorder [ADHD] medications) can be disregarded as judged by the Investigator.

9.7 Screen failures

Screen failures are defined as participants 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 participants. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

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

Re-screening can be performed once 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).
- Reserve participants not used in a previous group of participants.

For participants who are rescreened, a new screening number will be assigned and a new, signed ICF must be collected.

9.8 Participant withdrawal

9.8.1 General withdrawal criteria

Participants are free to voluntarily discontinue their participation in the trial, *i.e.*, withdraw consent, 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 in the eCRF.

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

Reasons for discontinuation can include:

- AE (as judged by the Investigator and/or Sponsor).
- Death.
- Logistical problem.
- Lost to follow-up.
- Non-compliance with study device.
- Non-compliance with trial drug.
- Non-compliance with trial schedule.
- Physician decision.
- Pregnancy.
- Protocol deviation.
- Technical problems.
- Withdrawal of consent (participant decision).
- Other.

9.8.2 Procedures for discontinuation of a participant from the trial

A participant 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 participant withdraws consent, the Investigator must ask the participant 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 11.3.1.16.

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.

9.8.3 Participant replacement

Participants who are prematurely withdrawn from the trial for any reason except the occurrence of AEs assessed as possibly or probably related to the trial treatment may be replaced.

9.9 Randomisation (Part A)

On Day 1 (Visit 2), participants in each cohort will be randomised in a 6:2 ratio to receive either GZ21T or placebo. Sentinel dosing will be applied for the first 2 participants in each cohort who will receive either GZ21T or placebo as randomised.

Randomisation will be performed in the EDC system, Viedoc™, employing static randomisation based on a computer-generated randomisation list generated using the statistical analysis software (SAS) Proc Plan (SAS version 9.4). The randomisation list will contain the participant number and treatment and will be kept by the randomiser in a sealed envelope until the database is locked. A copy of the randomisation list will be kept by an unblinded staff member at the site.

Sealed, individual treatment code envelopes will be kept in a locked and restricted area at the trial site for use in case of emergency unblinding. Code-breaking, if needed for iSRC evaluations or pharmacovigilance purposes (*e.g.*, assessment of a potential suspected unexpected serious adverse reaction [SUSAR]), is also available for appointed persons through the eCRF.

9.10 Blinding

Part A of the trial is double-blinded. The allocation of treatments will not be disclosed until clean file has been declared and the database has been locked.

The IMP and the placebo are close to identical in appearance. The placebo will be masked in such a way that the participants and study staff will remain blinded during the trial. The unblinded person doing the IMP preparation will not be involved in any trial-specific assessments or evaluations. Details will be specified in a separate Blinding Plan.

Part B of the trial is open-label and therefore no blinding will be performed.

9.11 Emergency unblinding during the trial

The treatment code may only be broken by the Principal Investigator or a delegate in case of an emergency where knowledge of the treatment is necessary for proper medical management of the participant. The code-breaking procedure must be carefully documented. The site staff and the participants will still be blinded for the treatments (active treatment or placebo) to be administered to the subsequent participants in order to minimise bias.

For unblinding procedures in case of a potential SUSAR, refer to Section 11.3.1.15 or in case of iSRC unblinding request, refer to Section 8.3.5.

10 TRIAL TREATMENTS

10.1 Identity of investigational medicinal products

GZ21T cream, [redacted].

Placebo for GZ21T cream, 0 mg/g, [redacted].

10.2 Manufacturing, packaging, labelling and release

All manufacturing, packaging, labelling and release of IMP will comply with applicable good manufacturing practice (GMP) requirements [12] and labelling requirements specified in Annex VI of Regulation European Union (EU) 536/2014 [13].

The manufacturer of the IMP is [redacted]. The IMP will be packed, labelled, and released by [redacted] and shipped directly to the trial site (CTC, Uppsala, Sweden) by a good distribution practice (GDP) qualified courier.

The unblinded site pharmacist/nurse at CTC holds the responsibility for receiving IMP deliveries, ensuring the maintenance of IMP quality during transportation, and validating that the IMP delivery aligns with the specifications outlined in the agreement with the Sponsor. Document verification, quality checks, storage condition confirmation, package integrity check and visual inspection of the IMP will be performed upon reception of the IMP in accordance with CTC's standard operating procedures (SOPs). Any deviations identified during reception should be reported to the Sponsor and/or its representative as applicable. The confirmation of receipt will be sent by the site-responsible personnel to the clinical research assistant and/or the Sponsor.

10.3 Conditions for storage

The IMP will be stored in an access-controlled storage area, restricted to unblinded site personnel, at CTC. The IMP will be stored at room temperature (15ºC – 25ºC). Automatic temperature logs are kept for all spaces where the IMP is stored.

10.4 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 CTC. There will be 2 unblinded persons working together, 1 person will handle the IMP and perform the preparation according to the randomisation list and the other person will supervise the process. The preparation and administration of IMP doses will be conducted within the timeframe during which the IMP quality remains consistently upheld as outlined in the IMP Manual.

CTC 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 participant, 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 participant must be accounted for.

10.5 Treatment administration

Participants will be administered a single dose of GZ21T or placebo as randomised and the area and application site will depend on the cohort. There are no fasting requirements prior to IMP application.

The cream will be applied as follows:

[redacted]

Before application, the treated area should be cleaned onsite with a gentle cleanser, then rinsed and patted dry. If needed, the treated area should be trimmed. The treated area will be marked with a medical skin waterproof marker. The IMP should not be applied to abraded, irritated or erythematous skin and application to skin folds should be avoided. During that time, participants should remain still without touching the treated area. Before treatment on Day 1, participants must have abstained from using moisturising ointments, creams, or emollients for 24 hours on the area to be treated. The IMP application date and time point (start and stop time) should be registered in the eCRF. For details, refer to the Pharmacy manual.

10.6 Continuation of treatment with investigational medicinal product

This is a Phase I trial in healthy volunteers who will receive no medical benefit from the treatment and thus there will be no treatment with GZ21T after the end-of-trial participation.

10.7 Treatment compliance

All IMP will be administered at the trial site under medical supervision to ensure compliance.

10.8 Return and destruction of investigational medicinal product

Any unused trial medication and all empty containers will be destroyed at the trial site upon confirmation from the Sponsor. The Monitor will perform the 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 8.1-1 and Table 8.1-2).

11.1 Recording of data

The Principal Investigator 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 the Sponsor in the eCRF and all required reports.

It is important that PK blood sampling occurs as close as possible to the scheduled time. In order to achieve this, the timing priority order at a particular time point is:

1. Blood samples for PK
2. Vital signs
3. Standard 12-lead safety ECG
4. Local tolerability

Safety laboratory samples will, for practical reasons, be taken in association with blood sampling for PK.

Time points for PK blood sampling, safety laboratory samples, 12-lead ECG and vital signs are outlined in Table 8.1-1 and Table 8.1-2. The actual time for PK sampling must not deviate more than ±10% from the planned time. For further details regarding time windows for PK sampling, refer to Section 11.4.1.

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.

11.2.2 Eligibility criteria

Eligibility criteria should be checked at the screening visit and verified before randomisation 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: sex, age, ethnicity, and race.

11.2.4 Height, weight, and body mass index and body surface area

Weight and height will be measured without shoes. BMI will be calculated, with 1 decimal, from the recorded height and weight. BSA will be calculated, with 2 decimals, from the recorded height and weight using a BSA conversion table.

11.2.5 Medical/surgical history

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

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

11.2.6 HIV and Hepatitis B/C

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

11.2.7 Pregnancy test

All WOCBP will do a pregnancy test at the screening visit (blood/plasma) and visits specified in Table 8.1-1 and Table 8.1-3 (urine dipstick).

11.2.8 Urine drug-screen

Urine will be screened for drugs of abuse at time points outlined in the schedule of events (Table 8.1-1 and Table 8.1-3) using the Drug-Screen Multi-15 Dip Test. Additional random tests can be performed during the trial period.

11.2.9 Alcohol test

An alcohol test will be performed at time points outlined in the schedule of events (Table 8.1-1 and Table 8.1-3). Additional random tests can be performed during the trial period.

11.2.10 Baseline symptoms

A baseline symptom is defined as an event that occurs between the participant's signing of the ICF and the administration of IMP (*i.e.*, an event that occurs during the screening period). Such events are not AEs and will be recorded as baseline symptoms in the AE log in the eCRF.

11.2.11 Prior and concomitant medication

Prior medications taken within 2 weeks prior to the first IMP administration will be obtained by participant interview in order to verify that the eligibility criteria are met (see also Section 9.6.2).

Medications are classified as prior if the stop date was before or on the day of the dose administration (pre-dose) and as concomitant if ongoing on the day of the dose administration, stopped after the dose administration or started after the dose administration. To distinguish between prior and concomitant medications on Day 1 (*i.e.*, the dosing day) 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 last end-of-trial visit must be documented appropriately in the participant'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 Assessments related to primary endpoints

11.3.1 Adverse events

The Principal Investigator 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 SOPs regarding emergencies and Phase I trials.

AEs will be handled in accordance with applicable regulations and guidelines [13,14].

For the purpose of this trial, AEs will be assessed in relation to the IMP.

11.3.1.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a participant 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.

11.3.1.2 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 ARs implies a reasonable possibility of a causal relationship between the adverse event and the IMP.

11.3.1.3 Definition of serious adverse event

A 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
- results in 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 convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.

11.3.1.4 Definition of serious adverse reaction

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

11.3.1.5 Definition of suspected unexpected serious adverse reaction

A SAR, the nature, severity, or outcome of which is not consistent with the reference safety information (IB for an unapproved IMP, Summary of Product Characteristics for an approved medicinal product used in accordance with the terms of the marketing authorisation).

11.3.1.6 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the start of IMP administration until the end-of-trial visit.

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

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

Investigators will not be obliged to actively seek AEs or SAEs after the conclusion of the trial participation. However, if the Investigator learns of any SAE, including 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.

11.3.1.7 Collection of adverse events

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

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

11.3.1.8 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 IMP, action taken, and outcome. 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.

11.3.1.9 Assessment of seriousness

The Investigator must assess and document the seriousness (serious or non-serious) of each AE using the definitions in Section 11.3.1.3. If the event is assessed as serious it must be reported as an SAE by the Investigator to the Sponsor according to Section 11.3.1.14.

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 participant 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.

11.3.1.10 Assessment of intensity

The grading of the intensity of AEs will follow the CTCAE v5.0 [7]. 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, undressing, feeding self, using the toilet, taking medications, and not being bedridden.

11.3.1.11 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the use of the IMP, using the definitions below. Each assessment should be recorded in the AE log of the eCRF.

Not related There is no evidence of causal relationship with the IMP.

Unlikely related The event has little evidence of causal relationship to the IMP.

Possible related The event has a suggestive causal relationship to the IMP, and an alternative aetiology is equally or less likely.

Probable related The event has a strong causal relationship to the IMP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely.

Definitely related The event is clearly related to the IMP.

An AE is considered causally related to the use of the IMP when the causality assessment is probable or possible.

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

11.3.1.13 Action taken with trial treatment

The Investigator must document the action taken with trial treatment using the definitions below and record it on the AE log of the eCRF.

Dose increased N/A

Dose not changed

Dose reduced N/A

Drug interrupted

Drug withdrawn N/A

Not applicable

Unknown

11.3.1.14 Reporting of serious adverse events

The Investigator must report SAEs within **24 hours** of awareness to the Sponsor or its designee, 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 eCRF. When the Investigator classifies the event as "serious" in the eCRF, and signs off the event, an automatic e-mail alert is sent to the Sponsor or its designee, and any other predefined recipients.

The backup procedure for reporting an SAE in case the eCRF is unavailable will be via the paper SAE form provided in the Investigator Site File (ISF). The Investigator must fill in the SAE form and send it Sponsor or its designee via secure file transfer. The trial site must notify the site, Monitor, via phone or e-mail about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE must be reported electronically as well.

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

The SAE report will be reviewed by the CTC's Pharmacovigilance department to ensure that the report is valid. The Sponsor or its designee will 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 11.3.1.15.

The Sponsor will perform an independent assessment of causality and provide a rationale for the assessment. 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 in the report. The Sponsor will perform an assessment of expectedness based on the applicable reference safety information (*i.e.,* in the IB for GZ21T).

If any additional information or documentation (*e.g*., autopsy report) on the SAE is required for the Sponsor's assessment of the SAE, the Sponsor or its designee 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 participant. 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. If the SAE report in the eCRF is updated, a new automatic e-mail alert is sent to the Sponsor or its designee.

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

11.3.1.15 Reporting of suspected unexpected serious adverse reactions

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

Only SUSARs on which the treatment allocation of the participant is unblinded should be reported to the Member State. When an SAE may be a SUSAR, the blind will be broken by the Sponsor or its designee for the specific participant prior to reporting and a new assessment will be made to determine if the reporting rules for SUSAR apply.

SAEs which are assessed by the Sponsor to be a SUSAR will be reported by the Sponsor or its designee to the EudraVigilance database 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 its designee, has received the information containing the minimum reporting criteria. The date will be documented on the acknowledgement receipt to the reporting Investigator.

The Sponsor, or its designee, is responsible for informing the Investigator at the trial site of relevant information about potential SUSARs. Investigators should only receive blinded information unless unblinded information is judged necessary for safety reasons.

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

11.3.1.16 Treatment and follow-up of adverse events

Participants 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 participant 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 consultations.

11.3.1.17 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the trial treatment must be stopped immediately, and the participant should be withdrawn from the trial.

Pregnancy itself will not be regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up by the Investigator and the Sponsor and documented even after the participant was discontinued from the trial.

All pregnancies must immediately be reported by the Investigator to the Sponsor or designee using the Pregnancy Report Form provided in the ISF. Once known, information on the outcome of the pregnancy must also be reported to the Sponsor or designee using the Pregnancy Report Form.

All events of congenital abnormalities, birth defects and spontaneous miscarriages are SAEs and must be handled and reported as such as described in Section 11.3.1.14.

Pregnancies in female partners of male trial participants should also be followed according to the procedures described above.

11.3.1.18 Medication errors and uses outside what is foreseen in the protocol

A medication error is an unintended failure in the drug treatment process that leads to or has the potential to lead to, harm to the participant.

Medication errors and uses outside what is foreseen in this protocol, including misuse and abuse of the product, will be reported in the participant's medical records, and documented in the protocol deviation (PD) log. Any medication error, misuse and/or abuse of the drug product will also be recorded in the AE log of the eCRF (refer to Section 11.3.1.8).

11.3.1.19 Treatment of overdose

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

Overdosing is not likely to occur in this trial since all IMP will be administered by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures will be adopted as required.

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 participant's medical records and documented in the PD log.

No known antidotes are available in case of overdose with GZ21T.

There have been no reported cases of GZ21T overdose.

11.3.2 Local tolerability

The Investigator will assess the incidence of local tolerability reactions of the administration site by direct inspection at visits and timepoints specified in Table 8.1-1 and Table 8.1-2 for Part A and in Table 8.1-3 for Part B. The assessment will include the Investigator's (or delegate's) evaluation of erythema, swelling, pruritus, burning and discoloration and dryness.

The presence/absence and intensity of local tolerability reactions at each time point will be recorded in a local tolerability module of the eCRF. Assessment of intensity will be performed as follows:

- Local tolerability reactions will be assessed by the Investigator or delegate as non, mild, moderate, or severe based on the definition of the reaction as outlined in Table 11.3-1. Discoloration is defined as change in skin tone other than erythema, *e.g.*, increased pigmentation or blanching, other than colour from the IMP. The intensity of each reaction will be documented in the eCRF.

Local tolerability reactions will be recorded as IMP-related AEs in the AE Log of the eCRF as follows:

- Erythema, swelling, pruritus, burning, blistering and urticaria: Grade 2 (moderate) and Grade 3 (severe) reactions **OR** Grade 1 if present ≥ 24 hours after the start of IMP application.
- Dryness and discoloration: Grade 2 (moderate) and Grade 3 (severe) reactions.

As for other AEs, the full duration of the reaction(s), and the highest recorded intensity per reaction, should be entered.

Table 11.3-1 Local tolerability evaluation scale

Reaction	None=0	Mild=1	Moderate=2	Severe=3
Erythema	No detectable erythema; skin of normal colour	Slight pinkness present	Definite redness, easily recognised	Intense redness
Swelling	No swelling	Mild	Moderate	Severe
Pruritus	No pruritus	Occasional, slight itching/scratching	Constant or intermittent itching/scratching that is not disturbing sleep	Bothersome itching/scratching that is disturbing sleep
Burning	No burning	Slight warm, tingling sensation; not really bothersome	Definite warm, tingling sensation that is somewhat bothersome	Hot, tingling/stinging sensation that has caused definite discomfort
Blistering	None	Discrete signs	Moderate	Severe
Urticaria	None	Discrete signs	Moderate	Severe
Discoloration	None	Mild	Moderate	Severe
Dryness	None	Mild	Moderate	Severe

11.3.3 Vital signs

Systolic and diastolic blood pressure and pulse will be measured in a 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.

11.3.4 Electrocardiogram

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

Safety ECGs will be reviewed and interpreted onsite 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.

11.3.5 Safety 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, Sweden, and analysed by routine analytical methods.

The safety laboratory parameters are defined in Table 11.3-2 and will be assessed at visits and time points specified in Table 8.1-1.

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 11.3-2 Safety laboratory parameters

Category	Parameter
Clinical chemistry	Alanine aminotransferase (ALT)
	Albumin
	Alkaline phosphatase (ALP)
	Aspartate aminotransferase (AST)
	Bilirubin (total and conjugated)
	Calcium
	Creatinine (estimated glomerular filtration rate [eGFR] included)
	Glucose
	Phosphate
	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])
Follicle-stimulating hormone (FSH) test	FSH (at the screening visit, females only for confirmation of menopause)
Pregnancy test (WOCBP only)	Plasma pregnancy (human chorionic gonadotropin [hCG]) test at screening
	Urine pregnancy test

11.3.6 Physical examinations

A complete physical examination will be performed at the screening visit and will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

A symptom-directed physical examination will be performed at Day 1 pre-dose and at the follow-up visits and will only be performed on the organ systems which are warranted, *e.g.,* when the participant has indicated any problems with the affected system or for which the Investigator or site nurse has reason to believe there may be a problem with the affected organ system.

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.

11.3.7 Cream absorption evaluation (Part B)

The cream absorption will be documented by photography of the administered area at timepoints specified in Table 8.1-4.

In addition, the cream absorption will be assessed at 30 minutes, 1 hour, and 2 hours post-administration on a 4-point scale scale:

"1= not absorbed"; "2= somewhat absorbed"; "3= mostly absorbed"; "4= completely absorbed"

The time until adequate abruption will be measured up to 2 hours after administration.

The Sponsor and the Investigator will determine if more cohorts will be explored at a higher or lower dose in order to reach the optimal dose/area or if the abosorption is sufficient.

11.4 Assessments related to secondary endpoints

11.4.1 Pharmacokinetic sampling and analysis (Part A)

Venous blood samples (approximately 4 mL) for the determination of plasma concentrations and PK characterisation of GZ21T after administration of the IMP, will be collected through an indwelling venous catheter or by venepuncture at the pre-specified visits and time-points detailed in Table 8.1-1 and Table 8.1-2.

The actual time for blood PK sampling must not deviate more than ±10% from the planned time. The pre-dose PK sample may be taken within 60 minutes prior to dosing.

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 diluted in acetonitrile, mixed using a vortex mixer and frozen at ≤ -80°C until shipment. Further details will be described in a separate laboratory manual.

Plasma samples for determination of plasma concentrations of GZ21T will be analysed by Scantox, Gothenburg, 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.

11.5 Appropriateness of measurements

All methods used are commonly used in standard medical care and in Phase I clinical trials. Non-compartmental analysis of PK parameters is standard for Phase I clinical trials.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.4.1.

Blood samples are collected according to standard procedures.

12.2 Volume of blood

The anticipated volume of blood samples collected during the trial from each participant will be approximately 132 mL in Part A and 24 mL in Part B (Table 12.2-1 and Table 12.2-2, respectively). For reference, a regular blood donation consists of between 350 mL to 450 mL (±10 %) for persons weighing at least 45-50 kg [15].

Table 12.2-1 Estimated blood volumes (Part A)

Assessment	Estimated number of sampling occasions	Estimated volume per occasion (mL)	Total (mL)
PK blood sampling	12	4 mL	48 mL
HIV, Hepatitis B/C	1	4 mL	4 mL
Clinical chemistry, haematology, coagulation	4	20 mL	80 mL
		Total:	**132 mL**

Table 12.2-2 Estimated blood volumes (Part B)

Assessment	Estimated number of sampling occasions	Estimated volume per occasion (mL)	Total (mL)
HIV, Hepatitis B/C	1	4 mL	4 mL
Clinical chemistry, haematology, coagulation	1	20 mL	20 mL
		Total:	**24 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).

Any remains from the safety laboratory samples will be disposed of after analyses.

The samples for analyses of PK parameters will be stored at ≤ -80°C until analysed. The samples will be disposed of after the clinical trial report (CTR) has been finalised.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their life-cycle.

CTC keeps full traceability of collected biological samples from the trial participants 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 life-cycle of the samples through internal procedures, monitoring of the trial site and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

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

The Principal Investigator will ensure that:

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

The Sponsor will ensure that the laboratory holding the samples is informed about the withdrawn consent immediately 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 ICH E6(R2) guideline [16].

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 while maintaining overall trial oversight:

- Implementing and maintaining quality assurance (QA) and quality control (QC) systems with written SOPs with regard to the management of identified risks, CTP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.
- Securing agreements with involved subcontractors and performing regular subcontractor oversight to ensure CTP compliance, GCP compliance and compliance with applicable regulatory requirements.
- Implementing a risk-based validated EDC system and maintaining 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 [17] and are consistent with the ICH E6 (R2) guideline for GCP [16], Regulation (EU) 536/2014 [13], and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The Principal Investigator is responsible for submission of the CTP, the participant information and ICF any other written information to be provided to the participants and any advertisements used for recruitment of participants to applicable IEC for authorisation.

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

Authorisation must be obtained in writing from both the IEC and Member State before the first participant can be recruited.

The Sponsor will provide the Member State, IEC, and Principal Investigator 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 Participant information and consent

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

The information will include the nature, objectives, benefits, implications, risks and inconveniencies of the trial, as well as the conditions under which the trial is to be conducted and the expected duration of the participant's participation. It will be emphasised that participation in the trial is voluntary and that the participant may withdraw from participation at any time and for any reason, without any detriment and without having to provide any justification. All participants 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 participant and by the Investigator. A copy of the participant information including the signed ICF will be provided to the participant.

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

The final authorised version of the participant information and ICF must not be changed without authorisation from the Sponsor and the applicable IEC.

14.4 Participant information card

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

- That they are participating in a clinical trial.
- Participant 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 Participant privacy and data protection

The clinical personnel affirm and uphold the principle of the participant'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) [18], these pseudo-anonymised data will not identify any persons taking part in the trial. If any part of the data, including biological samples, is handled by any other organisation, inside or outside the EU, appropriate agreements, setting out each one's respective roles and responsibilities, and how individuals may exercise their rights in respect of the data other documentation, such as transfer agreement, will be established, and verification of organisation quality and process systems will take place 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 participant 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 competent authority, have direct access to their medical records for verification of clinical trial procedures. For further details on the participant information and ICF process, refer to Section 14.3.

The participant 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 [18] and the request will be raised to the Principal Investigator.

The Investigator must file a participant 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 participant ID and will be processed by the Sponsor and other involved parties during the trial. After the trial ends, 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 is the data processor. Any subcontractors used in the trial are also data processors.

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

14.6 Data security breach

In order to avoid or minimise data security breaches, organisational, physical and technological security measures with a risk-based approach have been taken for all computer systems used to process data and personal data in this trial.

In the event of a data security breach, the controller (the Sponsor for trial data and the trial site for medical data):

- documents any personal data breaches, comprising the facts relating to the personal data breach, its effects and the remedial action taken.
- notifies the personal data breach supervisory authority, unless the data breach is unlikely to result in a risk to the rights and freedoms of natural persons.
- communicates the personal data breach to the data subject when the personal data breach is likely to result in a high risk to the rights and freedoms of natural persons.

14.7 Changes to the authorised clinical trial protocol

Any proposed change to the authorised final CTP, including appendices, will be documented in a written and numbered clinical protocol modification form. All substantial modifications to the protocol must be authorised according to applicable regulations before implementation.

14.8 Audits and regulatory inspections

Authorised representatives of the Sponsor, a regulatory authority, or an IEC may perform audits or regulatory 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 whether 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 Member State about an inspection at the trial site.

This trial may be subject to internal or external monitoring, auditing, or inspection procedures to ensure adherence to GCP. Access to all trial-related documents including direct access to source data will be given at that time.

14.9 Insurance

Participants will be covered under the Sponsor’s liability insurance policy through Acord. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating participants are also protected in accordance with national regulations, as applicable.

CTC has a company insurance covering services performed by CTC.

15 TRIAL MANAGEMENT

15.1 Training of trial site personnel

Before the inclusion of the first trial participant, a Sponsor representative or delegate will perform a site 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 the participating site, source data/documents, and reports for monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities. The extent and nature of the monitoring shall be determined by the Sponsor based on an assessment that takes into consideration all characteristics of the clinical trial.

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) the following:

- 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 participants.
- ensure that withdrawal of informed consent to the use of the participant'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 participant.
- 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 breaches and any data privacy breaches to the Sponsor.

Centralised monitoring will also be performed continuously by project team members at CTC 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 Principal Investigator and the Monitor to confirm the agreement before the start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the participant's medical history, and that verify the existence of the participant, the inclusion and exclusion criteria, and all records covering the participant's participation in the trial. They include laboratory notes, memoranda, material dispensing records, participant files, *etc*. The eCRF may constitute source data if clearly defined in the origin of the 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, Ankh Life Sciences Limited. The management and conduct of the clinical trial have been outsourced to the contract research organisation (CRO), CTC. The Principal Investigator is an employee of CTC.

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

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

The Principal Investigator must comply with all the terms, conditions, and obligations of the clinical trial agreement for this clinical trial. The participants will be compensated for the time spent participating in the trial. Travel expenses and/or loss of income will be reimbursed.

15.5 Trial timetable and end-of-trial

The trial is expected to start in Q2 2024 and to be completed by Q1 2025.

The start of the clinical trial is defined as the date of the first participant's first visit.

The Sponsor will notify each Member State of the start of a clinical trial (through the Clinical Trials Information System [CTIS]) within 15 days from the start of the clinical trial.

A participant 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 participant in the trial.

The Sponsor will notify each Member State of the end of the recruitment of participants for a clinical trial (through CTIS) within 15 days of the last participant's first visit.

The Sponsor shall notify each Member State of the end of the trial (through CTIS) within 15 days of the last participant's last visit.

15.6 Temporary halt or Early termination of the trial

The Investigator or the Sponsor may temporarily halt or terminate this trial prematurely for any reasonable cause.

A temporary halt for reasons not affecting the benefit-risk balance of the trial should be notified to the Member States concerned through CTIS within 15 days of the temporary halt. When the trial is resumed, a new notification should be sent within 15 days of the restart.

A temporary halt or early termination of a trial for reasons that change the benefit-risk balance (*e.g.,* safety reasons) should be notified to the concerned Member States through CTIS without undue delay, but no later than 15 days from the halt or termination. The reasons for the halt/termination and specific follow-up measures should be stated.

The restart of a trial following a temporary halt for reasons that change the benefit-risk balance requires authorisation of a substantial modification to the trial, see Section 14.7.

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

- There is sufficient ground that continuation of the trial will jeopardise participant health or safety.
- A decision by the Sponsor to suspend or discontinue the development of the IMP.

If the Member State obtains information that raises doubts about the safety or scientific validity of the trial, the Member State may also revoke the authorisation for, suspend, or require the Sponsor to modify aspects of the trial.

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

15.7 Reporting of serious breach

The Sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than 7 days of becoming aware of that breach.

15.8 Reporting of urgent safety measures

The Sponsor will notify, without delay but no later than 7 days from the date measures have been taken, each Member State is concerned about an urgent safety measure and the measures taken.

15.9 Reporting and publication

15.9.1 Registration in a publicly accessible database

The clinical trial will be registered in a publicly accessible and free-of-charge database (EU Clinical Trials Registry via CTIS) which is a primary or partner registry of, or a data provider to, the WHO International Clinical Trials Registry Platform (ICTRP) prior to first recruitment of any participants.

15.9.2 Public disclosure

Public disclosure of Sponsor and Member State documents and data (except data and documents related to quality) in the public database of CTIS takes place at the time of the decision by the Member State unless the Sponsor requests for a deferral of the public disclosure at the initial application when registering the clinical trial in CTIS.

The deferral for this trial will be according to the default time allowed for Category 1. No deferral is possible for the main characteristics of the clinical trial, conclusion part I, conclusion part II, outcome, and date of decision on clinical trial, start and end dates and temporary halt.

15.9.3 Summary of results and clinical trial report

A summary of the results of the trial accompanied by a layperson's summary of results will be submitted to the EU database within one year from the end of a clinical trial in all Member States concerned.

After completion of the trial, an ICH E3 [19] guideline-compliant CTR describing the conduct of the trial, 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 Principal Investigator, and the Sponsor.

Where the clinical trial was intended to be used for obtaining a marketing authorisation for the IMP, the applicant for the marketing authorisation shall submit to the EU database CTIS the CTR within 30 days after the day the marketing authorisation has been granted, the procedure for granting the marketing authorisation has been completed, or the applicant for the marketing authorisation has withdrawn the application.

15.9.4 Annual safety report

If the trial duration exceeds 1 year, the Sponsor must submit an annual safety report to the applicable Member States. 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 authorisation of the clinical trial.

15.9.5 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.

15.9.6 Publication

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

15.10 Archiving

The Sponsor will archive the content of the clinical TMF for at least 25 years after the end of the clinical trial [13]. The content of the clinical TMF shall be archived in a way that ensures that it is readily available and accessible, upon request.

Medical files of participants shall be archived in accordance with national law.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the site and filed in the ISF for archiving for 25 years after finalisation of the CTR. The ISF will also include any original source documents related to the trial, the participant identification list (providing the sole link between named participant source records and pseudonymous eCRF data) and the original signed ICFs. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

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 the eCRF, database setup and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information on discrepancies in the process. The database, data entry screens and programme will be designed in accordance with the CTP.

Data validation/data cleaning procedures are designed to assure the 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 the 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 the bedside (if the eCRF data constitutes source data). Source data are to be defined at the site before the inclusion of the first participant (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 participant.

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 participant's visit. To avoid inter-observer variability, every effort should be made to ensure that preferably the 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 the 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 the 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 in order to uniquely identify each sample record. File and data formats are agreed upon with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at CTC. 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 WHO anatomic therapeutic chemical (ATC) classification system.

16.7 Database lock

When all data have been entered and discrepancies solved, clean file will be declared, the database will be locked, the code will be broken, 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 (DBL).

The analyses of all endpoints will be performed by CTC.

17.1 General

Continuous data will in general be presented in terms of evaluable 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 part, cohort, treatment (active/placebo) and assessment time. Individual participant data will be listed by part, participant number, cohort, 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 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 administration of IMP. Details on potential imputation of missing data will be provided in the SAP.

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.

In Part A, approximately 64 participants will be screened to achieve 32 randomised participants.

In Part B, approximately 18 participants will be screened to achieve up to 9 included participants.

17.3 Analysis data sets

There will be 2 analysis sets defined in the trial. The full analysis set (FAS) will consist of all enrolled participants who received at least one dose of IMP (this set will also be used for all safety analyses). The PK analysis set (PKAS) will consist of all randomised participants in Part A who received at least one dose of IMP and provided an evaluable plasma concentration profile, and who have no AEs or PDs judged to affect the PK analysis. Individual PK values may be excluded from the analysis as specified in the SAP.

17.4 Missing, unused and spurious data

Methods for handling missing, unused and spurious data will be specified in the SAP.

17.5 Description of trial population

17.5.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight and height will be presented for all participants. All data will be listed by participant.

17.5.2 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 ATC levels 4 and 5.

All data will be listed by participant.

17.5.3 Investigational medicinal product use

Individual IMP use will be listed.

17.6 Analysis of primary endpoints

17.6.1 Adverse events

An overview of all AEs, including SAEs, intensity, relationship to IMP, and deaths will be presented by part, cohort, treatment, and overall. The incidence of AEs and SAEs will be summarised by SOC and PT by treatment, cohort and overall. An overview of any treatment-related (probably/possibly related) AEs will be summarised by SOC and PT and by treatment, cohort and overall if considered appropriate. All AE data will be listed by participant and include the verbatim term entered by the Investigator.

17.6.2 Local tolerability

An overview of all local tolerability reactions including maximum intensity grade per local tolerability reaction will be presented by part, cohort, treatment, and overall. Intensity grade for each local tolerability reaction will also be presented by cohort, treatment, and overall using a frequency table. Changes from baseline in intensity grade at each time point will be summarised in a shift table.

17.6.3 Vital signs

Vital signs will be summarised by treatment, cohort and overall. Data will be presented with absolute and percent change from baseline. All data will be listed by participant.

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 part, cohort, treatment, overall, and assessment time point using frequency tables. Changes over time will be presented using a shift table if considered appropriate. All measured ECG values will be summarised by treatment, cohort and overall, and assessment timepoint with absolute and percent change from baseline. All data will be listed by participant.

17.6.5 Safety laboratory

Safety laboratory data will be summarised by part, cohort, treatment, overall, and assessment time point with absolute and percent change from baseline. Abnormal, clinically significant values will be summarised separately if considered appropriate. All data will be listed by participant.

17.6.6 Physical examinations

Clinically significant and non-clinically significant abnormal findings will be specified and summarised by part, cohort, treatment, overall, and assessment time point. Changes over time will be presented using shift tables, if appropriate. All data will be listed by participant.

17.6.7 Cream abosorption (Part B)

Cream absorption measured on a 4-point scale will be presented using a frequency table by cohort using descriptive statistics. The time until adequate abruption will be summarised by cohort. All data will be listed by participant.

17.7 Analysis of secondary endpoints

17.7.1 Analysis of pharmacokinetics (Part A)

The PK analysis will be based on the PKAS and performed by CTC.

The following non-compartmental PK parameters will be assessed in the trial if sufficient data is available:

- AUC_{inf}
- AUC_{last}
- C_{max}
- T_{max}
- $T_{1/2.}$

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 the trial drug and then extrapolated to infinity using the concentration in the last quantifiable sample and the estimated terminal elimination rate constant ($Lambda_z$).

PK data will be presented by treatment and cohort using summary statistics with the number of measurements (n), arithmetic mean, SD, as well as median, minimum and maximum values. For all applicable PK parameters, the geometric mean and geometric coefficient of variation (CV%) will be presented. For the parameter T_{max}, the number of observations, and median, minimum and maximum values will be presented only.

All data will be listed by participant.

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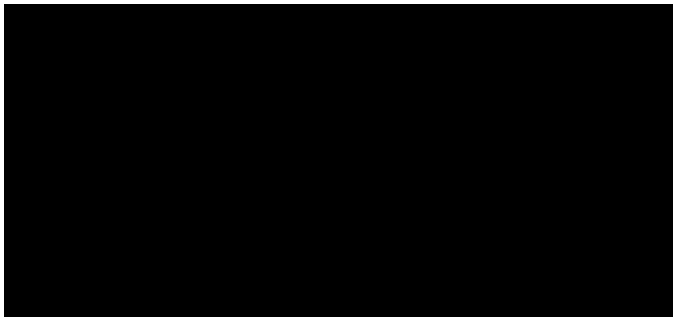
19 SIGNATURES

19.1 Approval of the clinical trial protocol

I, the undersigned, approve this CTP.

19.2 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.



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