Statistical Analysis Plan

Title:	**A Phase 2, single-center, randomised, double-blind, placebo-controlled, cross-over, cold challenge study investigating the effect of C21 on cold-induced vasoconstriction in subjects with Raynaud's Phenomenon (RP) secondary to systemic sclerosis (SSc)**
Short Title:	**Cold challenge with C21 in RP**
Sponsor:	**Vicore Pharma AB** **Kronhusgatan 11** **SE-411 05 Göteborg** **Sweden**
Trial ID:	**VP-C21-004**
NCT No.:	**NCT04388176**
EudraCT No.:	**2019-003203-35**
Investigational Medicinal Product:	**C21**
Indication:	**RP secondary to SSc**
Phase:	**2**
Version:	**Final 1.0**
Date:	**01-Feb-2021**

STATISTICAL ANALYSIS PLAN

A Phase 2, single-center, randomised, double-blind, placebo-controlled, cross-over, cold challenge study investigating the effect of C21 on cold-induced vasoconstriction in subjects with Raynaud's Phenomenon (RP) secondary to systemic sclerosis (SSc)

Protocol:	VP-C21-004
SGS LS number:	BE-80-1903115
Development phase:	II
Sponsor:	Vicore Pharma AB
Analysis purpose:	Final analysis
SAP version number:	Final 1.0
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TABLE OF CONTENTS

PROTOCOL HISTORY

Protocol:		
Version or ID	**Date (ddMMMyyyy)**	**Impact of the changes on the statistical analysis**
Final 1.0	20AUG2019	NAP
Final 2.0	27AUG2019	NAP
Final 3.0	11OCT2019	NAP
Final 4.0	22OCT2020	NAP

Protocol amendments:		
Version or ID	**Date (ddMMMyyyy)**	**Impact of the amendment on the statistical analysis**
NAP		

This statistical analysis plan (SAP) only considers the last version of the protocol, and of the protocol amendments, as listed above.

LIST OF ABBREVIATIONS

ADaM	analysis data model
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	analysis of variance
ANCOVA	analysis of covariance
AST	aspartate transaminase
bpm	beats per minute
CI	confidence interval
CRF	case report form
DBP	diastolic blood pressure
DSMB	data safety monitoring board
DY	relative day
ECG	electrocardiogram
FAS	full analysis set
GGT	gamma-glutamyl transferase
GMR	geometric mean ratio
HR	heart rate
ICF	informed consent form
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
NAP	not applicable
PP	per protocol
QTc	corrected QT interval
QTcB	Bazett's corrected QT interval
QTcF	Fridericia's corrected QT interval
RND	all randomised subjects analysis set
SAP	statistical analysis plan
SAF	safety analysis set
SBP	systolic blood pressure

SCR	all screened subjects analysis set
SD	standard deviation
SE	standard error
SGS LS	SGS Life Sciences
SOP	standard operating procedure
TEAE	treatment-emergent adverse event
TLF	tables, listings and figures
VS	vital signs
WHO	World Health Organisation

DEFINITION OF TERMS

bias	The systematic tendency of any factors associated with the design, conduct, analysis and evaluation of the results of a clinical trial to make the estimate of a treatment effect deviate from its true value. Bias introduced through deviations in conduct is referred to as 'operational' bias. The other sources of bias listed above are referred to as 'statistical'.
case report form (CRF)	A printed, optical, or electronic document designed to record protocol required information to be reported to the sponsor for each trial subject.
display	Analysis table, listing or figure
phase	Interval of time in the planned conduct of a study associated with a specific purpose: for example, screening, treatment, follow-up.
round half to even tie-breaking rule	Convention to round values ending with 5 to the nearest even digit. This method treats positive and negative values symmetrically, and is therefore free of sign bias, which is not the case for the round half up tie-breaking rule. The round half to even rule is implemented in the SAS® ROUNDE function.

Examples:

Database value	Rounded value
-1.35	-1.4
-1.25	-1.2
1.25	1.2
1.35	1.4

significant digit	All digits of a number used to express it to the required degree of accuracy, starting from the first non-zero digit.
study drug	Pharmaceutical form of an active ingredient or placebo, being tested or used as a reference in a clinical study.

1. INTRODUCTION

This SAP describes the final statistical analysis to be performed for the VP-C21-004 (BE-80-1903115) study.

This SAP covers the efficacy, safety, and general characteristics parts of the statistical analysis. It specifies the analysis displays to be presented and describes the methods and procedures in a more elaborated way than in the statistical methods section of the protocol.

The interim / DSMB / other analysis is / are not in the scope of this SAP.

The statistical analysis will process and present the results following the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) standards, in particular the ICH-E3, ICH-E6, and ICH-E9 guidelines.

1.1 STUDY OBJECTIVES

The primary objective of this study is:

- To evaluate the effect of a single dose C21 200 mg o.d. on cold-induced vasoconstriction in subjects with Raynaud's Phenomenon (RP) secondary to systemic sclerosis (SSc).

The secondary objectives of this study are:

- To evaluate the safety of a single dose C21 200 mg o.d. in subjects with RP secondary to SSc.
- To evaluate the effect of a single dose C21 200 mg o.d. on finger temperature in subjects with RP secondary to SSc.

The exploratory objective of this study is:

- To evaluate the effect of a single dose C21 200 mg o.d. on relevant biomarkers.

1.2 STUDY DESIGN

This is a randomised, double-blind, placebo-controlled, cross-over cold challenge study. As shown in Figure 1, the subjects are divided into 2 groups randomly, for different treatment sequence (C21-Placebo or Placebo-C21).

They will come to the clinic for four visits:

- Visit 1: Screening
- Visit 2: study drug administration and cold challenge (3-21 days after Visit 1)
- Visit 3: study drug administration and cold challenge (3-7 days after Visit 2)
- Visit 4: End of trial (3-15 days after Visit 3 or after withdrawal from trial)

Each subject is expected to participate in the study for 11-51 days including a 3-21 days screening period.

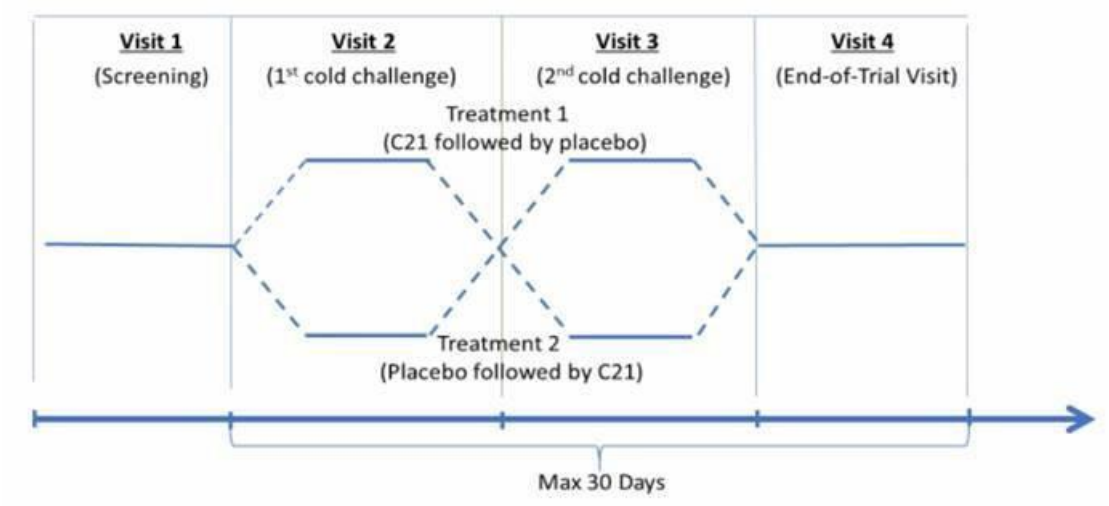

Figure 1. Trial Design

The schedule of assessments is in appendix 9.2.

1.3 EXPECTED SAMPLE SIZE

A total of 16 subjects was planned to be included. With 14 subjects a difference of 0.104 in the primary endpoint (log (AUC)) could be detected with a power of 90% (and alpha=0.1) based on variability previously reported, intra-individual standard deviation of the differences of 0.126 (Wilkinson et al 2018). Two additional subjects will be included to compensate for potential dropouts.

1.4 RANDOMISATION AND BLINDING

Eligible subjects will be randomised to receive either C21 or Placebo in a random order at Visit 2 and the opposite medication at Visit 3. The doses administered to each subject is:

- Placebo
- C21 200 mg

Each subject will be assigned a randomisation code number.

The study will be conducted in double-blind fashion and the allocation of treatment sequences will not be disclosed until the database has been locked.

1.5 INTERIM ANALYSIS

No interim analyses are foreseen.

1.6 SOFTWARE

SAS version 9.4 will be used for programming.

1.7 VALIDATION MODEL

SGS Life Sciences (SGS LS) – Clinical Research Statistics (STAT) standard operating procedures (SOPs) and work instructions (WIs) as effective at the project

start will be followed throughout the project, provided the applicable regulatory requirements are still met.

The primary endpoint tables/figures/listings will be validated according to Model C: review by an independent person and independent programming of the parameters indicated in this SAP. All other analysis tables/figures/listings will be validated according to Model B: review by an independent person (see SOP.STAT.020).

2. EFFICACY, PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

2.1 EFFICACY

2.1.1 Available data

The efficacy parameters are collected by thermography and capillaroscopy. The following parameters are available: Temperature (of each finger), Area Under the Curve (of each finger and the mean for all 8 fingers), Maximum skin temperature after rewarming (of each finger and the mean for all 8 fingers), Distal Dorsal Difference (of each finger and the mean for all 8 fingers), Gradient of rewarming (of each finger and the mean for all 8 fingers), Mean Velocity, Mean Width, Mean Shape, Mean Orientation, and Mean Density.

2.1.2.1 PRIMARY ENDPOINT

The primary endpoint is defined as the area under the curve for rewarming of each finger after cold challenge (AUC) which is measured by thermography and available in the clinical database provided by a third-party vendor as specified in the data transfer agreement.

AUC is calculated using the linear trapezoidal rule, by summing all individual trapezoids:

$$AUC_{t_i - t_{i+1}} = \frac{1}{2}(Temp_i + Temp_{i+1}) * (t_i - t_{i+1})$$

Log-transformation will be applied.

2.1.2.2 SECONDARY ENDPOINTS

The following secondary endpoints are available in the clinical database provided by a third-party vendor as specified in the data transfer agreement:

- Maximum skin temperature after rewarming post-cold challenge (MAX)
  - Pre-cold challenge time points are not considered.
- Distal dorsal difference (DDD): the mean difference in temperature between the dorsum and the finger for different time points
- Gradient of rewarming in the first 2 minutes post-cold challenge (GRAD):
  - $Gradient_{t_i - t_{i+1}} = \frac{Temp_i - Temp_{i+1}}{t_i - t_{i+1}}$

Log-transformation will be applied, except for DDD.

2.1.2.3 EXPLORATORY ENDPOINTS

Nailfold capillaroscopy exploratory endpoints (red blood cell Mean Width, Mean Shape, Mean Orientation, Mean Density, and Mean Velocity) are available in the clinical database provided by a third-party vendor as specified in the data transfer agreement.

Change in finger temperature from intake of IMP to start of cold challenge will be derived.

Potential analyses of biomarkers related to endothelium and platelets are not in scope of this SAP.

2.1.2 Inferential statistics

All statistical comparisons will be made using two-sided tests at the 0.1 significance level unless specifically stated otherwise. As this is a proof-of-concept study, no correction method for multiple testing is foreseen. P-values should thus be interpreted accordingly.

Different from the definition of baseline value in section 5.2.2, the baseline value of the period is defined uniquely for each endpoint in this section. The baseline values for the endpoints AUC, MAX, GRAD and change in finger temperature are the last thermography temperature per finger before IMP administration in each period. For the endpoint DDD, the baseline is the thermography DDD value before IMP administration in each period. Similarly, the Mean Velocity value before IMP administration is treated as baseline for the exploratory endpoint capillaroscopy Mean Velocity.

The modelling of the primary endpoint is based on the log-transformed value of AUC. Multiplicative ANCOVA model will be used, with patient (sequence), sequence, period, treatment and baseline value of the period as covariates. Treatment, sequence and period will be modelled as fixed effects and patient nested in sequence will be modelled as random effect. The default standard Variance Components covariance structure is preferred in the analysis.

The secondary efficacy endpoints will be compared between treatment groups using similar multiplicative ANCOVA models for MAX and GRAD, with the same covariates mentioned above. Therefore, log-transformation will also be applied for the endpoints MAX and GRAD respectively. For the endpoint DDD, a similar additive ANCOVA model with the same covariates will be used. Since the DDD data is collected every 10 mins before cold challenge test, we would model DDD at each measurement time point separately. The selection principle of covariance structure remains the same for the additive and multiplicative models of secondary endpoints.

Similar additive ANCOVA models will be used for the exploratory endpoints change in finger temperature and capillaroscopy Mean Velocity, with the same covariates from ANCOVA model of AUC. For capillaroscopy Mean Velocity, the data would be collected multiple times at different time points. More specifically, the before-cold-challenge and post-recovery value in each period will be treated as the response variables for 2 separate ANCOVA models of capillaroscopy Mean Velocity. The selection principle of covariance structure remains the same for all the models of exploratory endpoints.

SAS code can be found in section 9.1.

2.1.3 Presentation of results

A listing will be prepared showing all derived parameters for different efficacy endpoints.

2.1.3.1 PRIMARY ENDPOINT

For primary endpoint, descriptive statistics (n, mean, the standard error (SE), median, interquartile range, minimum, and maximum) will be calculated per treatment group in each period.

For multiplicative AN(C)OVA model, the model estimates will be back-transformed. A table will report the back-transformed least square means per treatment group with 90% CI, and geometric mean ratio with 90% confidence intervals and 2-sided p-value.

2.1.3.2 SECONDARY ENDPOINTS

All the secondary endpoints will be summarised by means of descriptive statistics (n, mean, the standard error (SE), median, interquartile range, minimum, and maximum) per treatment group per treatment and period. The results will also be presented at each time point for DDD.

Secondary endpoints will be analysed based on the same multiplicative AN(C)OVA model as for the primary endpoint described above except for the DDD endpoint. The results will also be back-transformed and reported in a similar way as mentioned above.

For DDD, separate tables will report the results of the respective AN(C)OVA models for each measurement time point, including the least square means per treatment group with 90% CI, least square mean differences (C21 - placebo) with 90% CI and 2-sided p-value for testing differences between treatment groups.

2.1.3.3 EXPLORATORY ENDPOINTS

The following parameters of Nailfold capillaroscopy will be listed: Mean Width, Mean Shape, Mean Orientation, and Mean Density.

For Mean Velocity, descriptive statistics (n, mean, the standard error (SE), median, interquartile range, minimum, and maximum) per treatment group will be reported at different time points in each period. A table will report the results of the additive ANCOVA models, including the least square means per treatment group with 90% CI, least square mean differences (C21 - placebo) with 90% CI and 2-sided p-value for testing differences between treatment groups.

3. SAFETY ANALYSES

3.1 ADVERSE EVENTS

3.1.1 Available data

Adverse events (AEs) are coded into system organ classes and preferred terms using the medical dictionary for regulatory activities (MedDRA). For each AE, start and stop datetimes are collected as well as severity, a seriousness flag, treatment-relationship, action taken towards the study drug and outcome.

3.1.2 Derivation rules

Treatment-emergent adverse events (TEAE) are defined as AEs starting on or after first administration of any study drug.

Based on their start datetime, AEs will be allocated to the phase and period during which they started. Each AE will therefore be reported in only one phase and period. Phases and periods are defined in section 5.2.1. In case the AE start datetime is incomplete or missing and the AE could consequently be allocated to more than one phase or period, a worst-case allocation will be done as detailed below:

- Treatment phase vs. non-treatment phase: AE will be allocated to the treatment phase unless the available parts of the AE start or stop datetime provide evidence for allocating to the non-treatment phase.
- Multiple treatment periods: AE will be allocated to the last treatment period unless the available parts of the AE start or stop datetime provide evidence the AE did not occur during that period or unless the period is a placebo period.

A fatal AE is defined as an AE with outcome ‘fatal’.

An AE for which the study drug was discontinued is defined as an AE with action taken ‘drug withdrawn’.

AE onset and duration will be calculated as follows when start and stop dates are fully known:

- AE onset day (vs. first administration) =
  - AE start date ≥ date of first administration: AE start date – date of first administration + 1 day
  - AE start date < date of first administration: AE start date – date of first administration
- AE onset day (vs. start of phase / period) = AE start date – analysis phase / period start date + 1 day
- AE duration (days) =
  - AE end date – AE start date + 1 day
  - date of last contact – AE start date + 1 day (when the AE start date is fully known but the AE is not resolved at the end of the study)

    In this case the duration will be presented as “>x days”.

3.1.3 Presentation of results

Tables will present TEAEs only. Pre-treatment AEs will only be listed.

An overview table by period will show the number and percentage of subjects with at least one event and the number of events for the following:

- TEAEs
- Serious TEAEs
- TEAEs by severity
- Fatal TEAEs
- Treatment-related TEAEs
- Serious treatment-related TEAEs
- TEAEs for which the study was discontinued

Summary tables by MedDRA system organ class and preferred term will show the number and percentage of subjects with at least one event. The table of TEAEs will additionally show the number of events. Each AE record in the clinical database is considered as a distinct adverse event and is counted as such.

Separate tables will be prepared for the following:

- TEAEs
- TEAEs by worst severity
- TEAEs by worst relationship to study drug

All AEs will be listed. Separate listings will be prepared for serious AEs, AEs for which the study was discontinued and fatal AEs.

3.2 CLINICAL LABORATORY EVALUATION

3.2.1 Available data

Per protocol, the following laboratory parameters are expected:

- Haematology: Haemoglobin (Hb), haematocrit (Erythrocyte volume fraction), platelet count (Thrombocyte particle concentration [TPC]), leucocyte count, Mean Corpuscular Volume (MCV)
- Biochemistry: Albumin, alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase Gamma (ALP), bilirubin, Blood Urea Nitrogen (BUN), calcium, creatinine, C-Reactive Protein (CRP), Gamma Glutamyl Transferase (gGT), glucose, Lactate dehydrogenase (LD), potassium, sodium
- Urinalysis: PH, glucose, protein/albumin and Hb

For each laboratory record, laboratory test, test result in standardised unit, sample datetimes, fasting status (if available), and clinical significance as assessed by the investigator are collected. Normal ranges are available as provided by the laboratory.

3.2.2 Derivation rules

The following abnormality categories will be defined:

- Low: value < lower limit of normal range
- Normal: lower limit of normal range ≤ value ≤ upper limit of normal range
- High: value > upper limit of normal range

Note:

- Classification will be done in standardised units, using non-imputed values and limits.

3.2.3 Presentation of results

The statistical analysis will present results in standardised units.

Continuous laboratory parameters will be summarised by means of descriptive statistics at baseline and at each scheduled post-baseline visit. Actual values and changes from baseline will be tabulated separately. Categorical parameters will be listed only.

Laboratory abnormalities will be presented as cross-tabulations of the abnormality at each scheduled post-baseline visit versus the baseline abnormality.

All laboratory data will be listed.

3.3 VITAL SIGNS

3.3.1 Available data

The following vital signs parameters are collected: pulse rate in supine position, systolic (SBP) and diastolic blood pressure (DBP) in supine position, body temperature (after 10 mins of rest).

For each vital sign record, parameter name, test result and unit, assessment datetimes and clinical significance as assessed by the investigator are collected.

3.3.2 Derivation rules

Abnormalities are defined in below table.

	Pulse rate (bpm)	**SBP (mmHg)**	**DBP (mmHg)**	**Temp (°C)**
Low	<50	<90	<60	<35.5
Normal	50-100	90-140	60-90	35.5- 37.5
High	>100	>140	>90	>37.5

3.3.3 Presentation of results

Vital signs parameters will be summarised by means of descriptive statistics at each visit as collected in the CRF (visit 1, 2, 3, and 4).

Abnormalities will be presented as cross-tabulations of the abnormality at the scheduled post-baseline visit 4 versus the visit 1 abnormality.

All vital signs data will be listed.

3.4 ELECTROCARDIOGRAMS

3.4.1 Available data

The following electrocardiogram (ECG) parameters will be collected: heart rate (HR), RR interval, QRS interval, PR interval, QT interval and corrected QT interval (Bazett / Fridericia).

For each ECG record, parameter name, test result and unit, assessment datetimes and clinical significance as assessed by the investigator are collected.

3.4.2 Derivation rules

In case triplicates value are available, mean values of the triplicates will be calculated per time point and rounded as detailed in section 5.3.3. Throughout the analysis, including the derivation of baseline and abnormalities, the mean values will be used. Individual triplicate values will only be listed.

If needed, HR can be calculated from the RR interval as follows: HR = 60 / (RR (in sec)).

If not available in the database, corrected QT intervals (QTc) will be calculated using Fridericia's (QTcF) and Bazett's (QTcB) formula specified below and rounded as detailed in section 5.3.3:

- QTcB (ms) = QT (ms) * $\sqrt{\frac{HR(bpm)}{60}}$
- QTcF (ms) = QT (ms) * $\sqrt[3]{\frac{HR(bpm)}{60}}$

Abnormalities for HR, QRS and PR parameters are defined in below table.

	HR (bpm)	PR (ms)	QRS (ms)
Low	<50	<120	<60
Normal	50-100	120-200	60-109
High	>100	>200	>109

For QTc interval (ms), the following categories are defined:

- Actual values:
  - ≤ 320 (Low)
  -]320; 450]
  - > 450 (High)

- Changes:
  - ≤ 30 (normal)
  -]30; 60]
  - > 60

3.4.3 Presentation of results

Uncorrected QT interval will only be listed, RR interval will not be displayed.

Continuous ECG parameters will be summarised by means of descriptive statistics at baseline and at each scheduled post-baseline visit. Actual values and changes from baseline will be tabulated separately.

Abnormalities of the actual values will be presented as cross-tabulations of the abnormality at each scheduled post-baseline visit versus the baseline abnormality.

Abnormalities of the QTc changes will be presented as tabulations of the change abnormality at each scheduled post-baseline visit.

All ECG data will be listed.

3.5 PHYSICAL EXAMINATIONS

Abnormal physical examination findings will be listed.

4. GENERAL CHARACTERISTICS ANALYSES

4.1 SUBJECT DISPOSITION

The following subject data will be tabulated:

- The number of subjects in each analysis set
- Dates of first signed informed consent, last visit and last contact (overall only)
- The number and percentage of subjects who completed or discontinued the study as documented on the study termination page and the number and percentage of subjects for each study discontinuation reason by actual treatment

All information collected in the CRF concerning allocation, code breaking, study discontinuation will be listed.

4.2 PROTOCOL DEVIATIONS AND ELIGIBILITY

All available information concerning major protocol deviations, violations on eligibility criteria and subjects not treated will be listed.

4.3 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

4.3.1 Available data

The following parameters will be available:

- Demographics: sex, age, height, body weight at screening, date of birth, date of signing informed consent form (ICF), date of first diagnosis of disease under study, and disease-specific scoring
- Screening tests: serology (HBsAg, HCVAb and HIV 1+ 2 AgAb), Thyroid Stimulating hormone (TSH), ethanol and urine drug screen, pregnancy tests, finger temperature
- Baseline disease characteristics: date of first diagnosis of systemic sclerosis (SSc) and disease-specific scoring (modified Rodnan skin score)

4.3.2 Derivation rules

The following parameters will be derived:

- Body mass index (BMI) at screening (kg/m²) = (body weight at screening (kg)) / (height (m))²

  Note: The BMI will be recalculated and rounded as detailed in section 5.3.3, even when already available in the database.
- Age category: < 65 years / ⩾ 65 years
- Baseline disease severity: <median of Raynaud's Condition score / ⩾ median of Raynaud's Condition score
- Time since diagnosis (years): (date of ICF – date of diagnosis)/365.25

  Note: Partially missing dates will be imputed as specified in section 5.3.2

4.3.3 Presentation of results

Demographics will be presented using descriptive statistics for age, height, body weight and BMI and frequency tabulations for sex, age category and baseline disease severity.

Baseline disease characteristics will be presented using descriptive statistics for time since diagnosis and disease-specific scoring.

All demographic data and baseline disease characteristics will be listed. Listings will also be created for screening tests and pregnancy tests.

4.4 MEDICAL HISTORY AND CONCOMITANT DISEASES

4.4.1 Available data

Medical history findings are coded using the medical dictionary for regulatory activities (MedDRA) into system organ classes and preferred terms. For each finding (MH.MHCAT is 'MEDICAL HISTORY'), a start and stop date or ongoing flag is collected.

4.4.2 Derivation rules

The following parameters will be derived:

- Medical history finding: not ongoing at screening (MH.MHENRTPT is 'BEFORE')
- Concomitant disease finding: still ongoing at screening (MH.MHENRTPT is 'ONGOING' or missing)

4.4.3 Presentation of results

Medical history and concomitant diseases will be tabulated separately. Each table will show:

- The number and percentage of subjects with and without findings
- The number and percentage of subjects with findings by system organ class and coded term

Medical history and concomitant diseases will be presented by treatment sequence and overall.

All medical history and concomitant diseases data will be listed separately.

4.5 PRIOR AND CONCOMITANT THERAPIES

4.5.1 Available data

Concomitant medication will be coded using the latest version of World Health Organisation (WHO) Drug Reference List. ATC selection is performed. ATC coding up to level 5 is available in the clinical database. For each therapy, a start date or prior flag and stop date or ongoing flag are collected.

4.5.2 Derivation rules

Therapies will be allocated into one of the following categories:

- Prior therapies: the therapy ended before the first study drug administration
- Concomitant therapies: the therapy was taken after the first study drug administration

Based on their start and stop datetime, concomitant therapies will be allocated to each period during which they were administered. A therapy can therefore be reported in more than one period.

Phases and periods are defined in section 5.2.1. Concomitant therapies with (partially) missing dates will be allocated to each period unless the available parts of the therapy start or stop datetime provide evidence the therapy was not taken during that period.

4.5.3 Presentation of results

Prior and concomitant therapies will be tabulated by ATC class (level 5) and generic term.

All prior and concomitant therapies data will be listed with detailed information about ATC classes. All prior and concomitant therapies data will be listed.

4.6 EXPOSURE TO STUDY DRUG

4.6.1 Available data

For each study drug administration, the start datetimes, dose, administration route and fasting status will be recorded.

4.6.2 Derivation rules

4.6.3 Not applicable. Presentation of results

All exposure data will be listed.

5. GENERAL METHODOLOGY

5.1 ANALYSIS SETS

5.1.1 Analysis sets

The following analysis sets will be considered in the statistical analysis:

All screened subjects set (SCR):	subjects who *signed an informed consent* to participate in this study
All randomised subjects set (RND):	subjects who were *randomised* into this study
Safety analysis set (SAF):	randomised subjects who were *exposed* to the study drug
Full analysis set (FAS):	all subjects/subjects who have been randomised and received at least one dose of IMP and who has at least one post-baseline assessment of efficacy data allowing endpoint computation from each of the 2 treatment periods.
Per protocol (PP) analysis set:	a subset of FAS and consists of all subjects in FAS without any major protocol deviations that are judged to compromise the analysis of the data. All protocol deviations will be judged as major or minor prior to database lock.

Notes:

- Having signed an informed consent is defined as having a complete informed consent signature date in the database.
- Randomised is defined as having a complete randomisation date in the database or any information to confirm randomisation.
- Being exposed to the study drug is defined as having an exposure date or any information confirming exposure present in the database.

Unless stated otherwise, the SAF will be used for the general characteristics and safety tables, listings and figures. The FAS will be used for the efficacy tables, listings and figures. In case the population differs by more than 2 patients from the safety population, demographic data table will be repeated for the FAS. The PP analysis set will be used for the sensitivity analysis of the primary efficacy endpoint.

5.1.2 As planned versus as actual analysis

For analyses done on the safety analysis set, the actual treatment sequence of the subject will be considered.

For all other analyses, the planned treatment sequence will be used.

5.2 PHASES, PERIODS AND TIME POINTS

5.2.1 Phases and periods

Phase	Period	Start	End
Screening		Date of signing the informed consent form (ICF), with 00:00 added as time part	First administration datetime in period 1 – 1 minute
Treatment	Period 1	First administration datetime in period 1	First administration datetime in period 2 – 1 minute
	Period 2	First administration datetime in period 2	Date of last contact, with 23:59 added as time part

Per definition, and for each subject, the first phase starts on the date of the earliest available ICF signature with 00:00 added as time part, and the last available phase ends on the date of last contact, with 23:59 added as time part.

All tables, figures and listings will present treatments rather than periods.

AEs and concomitant therapies will be allocated to phases and periods as described in sections 3.1.2 and 4.5.2 respectively.

5.2.2 Baseline and change from baseline

For baseline disease characteristics, clinical laboratory evaluation, and electrocardiograms, the baseline value is the last available and non-missing value before the first administration of study drug. This definition does not apply in the efficacy part, which has its own baseline definition in part 2.1.2.

Change from baseline is defined as:

Change from baseline at time point t = value at time point t – baseline value.

5.2.3 Relative day

Relative days (DY) will be calculated according to the following rule:

- Concerned date < reference date: DY = concerned date – reference date
- Concerned date ≥ reference date: DY = concerned date – reference date + 1

The reference date is the date of first administration of study drug.

5.2.4 Analysis visits

For clinical laboratory evaluation and electrocardiograms, the baseline value is the last available and non-missing value before the first administration of study drug. This value corresponds to Visit 1, except in case of retesting. Reason for this approach is the use of retest results for subject eligibility assessment.

For all other assessments after the reference date, the analysis will use the visits and time points indicated on the subject's case report form (CRF). Unscheduled assessments after the reference date will only be listed.

Baseline is defined in section 5.2.2.

5.3 IMPUTATION AND ROUNDING RULES

5.3.1 Missing values

No imputation will be done of missing values (i.e. observed cases analysis).

5.3.2 Handling partially or completely missing dates in calculations

No imputation will be done of missing values (i.e. observed cases analysis). If missing date information causes a conflict for phase allocation, a worst case/conservative approach will be implemented.

5.3.3 Rounding of derived variables

Derived variables will be rounded to the appropriate number of significant digits (see Definition of terms):

- Corrected QT intervals will be rounded to integer.
- Mean of triplicates, mean scores and BMI will be rounded to 1 decimal.
- Log-transformed values will not be rounded.
- Ratios will be rounded to the number of significant digits of the parameter with the least number of significant digits.
- When converting values to different units, the converted value will have the same number of significant digits as the original value, limiting the number of decimals to 3.

  Examples:

Parameter	Database value	Converted value
GFR	52 mL/min	0.87 mL/s
Creatinine	0.71 mg/dL	63 µmol/L
Urate	4.7 mg/dL	280 µmol/L

All derivations will be done before rounding. Rounding will be done using the round half to even tie-breaking rule (see Definition of terms).

5.3.4 Outliers

There will be no outlier detection. All measured values will be included in the analyses.

5.4 PRESENTATION OF RESULTS

5.4.1 Calculation of descriptive statistics and percentages

For continuous parameters, full descriptive statistics will only be presented if there are at least 2 non-missing observations. Alternatively, only the number of non-missing data points and mean are shown.

Descriptive statistics will include the number of non-missing data points, the arithmetic mean, the standard deviation (SD) and standard error (SE), the median, minimum and maximum.

Mean, median, SE, SD and CI will be presented with one more decimal place than the individual values. Minimum and maximum will be presented with the same number of decimal places than the individual values.

For frequency tabulations and cross-tabulations, missing values will not be included in the denominator count when computing percentages. For cross-tabulations of post-baseline results versus baseline results, a 'missing' category will be shown for baseline results if applicable.

Percentages will be shown with one decimal place.

5.4.2 Presentation of treatments

The following treatment labels will be used in the tables, listings and figures:

- C21: C21 200mg
- PLA: Placebo

When presented by treatment sequence:

- C21-PLA
- PLA-C21

If possible, the long labels (i.e. C21 200mg or Placebo) should be used for tables, listings and figures presented by treatment. If needed for layout purposes, the short labels (C21 or PLA) will be used instead.

Treatment sequences will be presented using the short labels for tables and listings (e.g. C21-PLA).

In the general characteristics' analysis, a grand total will be added to summarise all subjects over sequences / treatments. Grand totals will be shown last.

5.4.3 Ordering in tables, figures and listings

In the analysis of general characteristics, results will be presented by sequence except where stated otherwise. For efficacy and adverse events, results will be presented by treatment.

All listings will be ordered by treatment sequence, subject, analysis visit and time point, unless specified otherwise.

In tables and figures showing several parameters, each parameter will begin on a new page and parameters will be sorted alphabetically, within the parameter category if applicable.

6. CHANGES TO THE PLANNED ANALYSIS

6.1 CHANGES NOT COVERED BY PROTOCOL AMENDMENTS BEFORE DATABASE LOCK

6.2 CHANGES NOT COVERED BY PROTOCOL AMENDMENTS AFTER DATABASE LOCK

6.3 CHANGES TO THE FINAL STATISTICAL ANALYSIS PLAN

SAP version number	SAP version Date (ddMMMyyyy)	Changes

7. REFERENCES

ICH Topic E6(R2) Guideline for Good Clinical Practice – Step 4, 9 November 2016.

An analysis of the time-relations of electrocardiogram. Bazett HC Heart 1920 7:353–370.

Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. Fridericia LS Acta Med Scand 1920 15:469–485.

Which QT correction formulae to use for QT monitoring? Vandenberk B, Vandael E, Robyns T et al. J Am Heart Assoc. 2016;5:e003264.

ICH guideline E14: the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs (R3) – questions and answers, January 2016.

ICH Topic E9 Statistical Principles for Clinical Trials – Step 5 – Note for Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96), September 1998.

Hughes M, Snapir A, Wilkinson J, Snapir D, Wigley FM, Herrick AL. Prediction and impact of attacks of Raynaud's phenomenon, as judged by patient perception. Rheumatology (Oxford) 54:1443-7, 2015

8. LIST OF TABLES, LISTINGS AND FIGURES

8.1 TABLES

LABORATORY DATA		
14.3.2.1	Descriptive Statistics of Laboratory Test Actual Values	SAF
14.3.2.2	Descriptive Statistics of Changes From Baseline in Laboratory Test Results	SAF
14.3.2.3	Cross-Tabulation of Laboratory Abnormalities Versus Baseline	SAF
VITAL SIGNS		
14.3.3.1	Descriptive Statistics of Vital Signs Actual Values	SAF
14.3.3.2	Cross-Tabulation of Vital Signs Abnormalities Versus Visit 1	SAF
ECG		
14.3.4.1	Descriptive Statistics of 12-lead ECG Actual Values	SAF
14.3.4.2	Descriptive Statistics of Changes From Baseline in 12-lead ECG	SAF
14.3.4.3	Cross-Tabulation of 12-lead ECG Abnormalities Versus Baseline	SAF
14.3.4.4	Cross-Tabulation of 12-lead ECG QTc Change Abnormalities Versus Corresponding Actual Value	SAF
14.3.4.5	Tabulation of Holter ECG Results	SAF

8.2 LISTINGS

GENERAL CHARACTERISTICS		
16.2.1.1	Allocation	RND
16.2.1.2	Code Breaking Information	RND
16.2.1.3	Analysis Phases and Periods	SAF
16.2.1.4	Study Discontinuation	SAF
16.2.2.1	Protocol Deviations	SAF
16.2.2.2	Violations on Eligibility Criteria	SAF
16.2.2.3	No-Treatment Subjects	SCR minus SAF
16.2.4.1	Demographic Data	SAF
16.2.4.2	Baseline Disease Characteristics	SAF
16.2.4.3	Screening Tests	SAF
16.2.4.4	Pregnancy Tests	SAF
16.2.4.5	Medical History	SAF
16.2.4.6	Concomitant Diseases	SAF
16.2.4.7	Prior and Concomitant Therapies	SAF
16.2.4.8	Comments	SAF
16.2.5.1	Exposure to Study Drug	SAF
16.2.5.2	Cold Challenge	SAF
EFFICACY		
16.2.6.1	Efficacy: Thermography Endpoints	FAS
16.2.6.2	Efficacy: Nailfold Capillaroscopy Endpoints	FAS

SAFETY

ADVERSE EVENTS

16.2.7.1	Adverse Events	SAF
16.2.7.2	Serious Adverse Events	SAF
16.2.7.3	Fatal Adverse Events	SAF
16.2.7.4	Treatment-Emergent Adverse Events Resulting in Study Discontinuation	SAF

PHYSICAL EXAMINATIONS

16.2.7.5	Physical Examinations Abnormalities	SAF

LABORATORY DATA

16.2.8.1	Laboratory Test Results	SAF

VITAL SIGNS

16.2.9.1	Vital Signs Results	SAF

ECG

16.2.10.1	12-lead ECG Results	SAF
16.2.10.2	12-lead ECG Interpretation and Morphology	SAF
16.2.10.3	Holter ECG Interpretation and Morphology	SAF

8.3 FIGURES

14.2.1.1	Histogram of Primary Endpoint Thermography AUC	FAS
14.2.1.2	Histogram of Primary Endpoint Thermography AUC	PP
14.2.1.3	Scatter Plot of Primary Endpoint Thermography AUC	FAS
14.2.1.4	Scatter Plot of Primary Endpoint Thermography AUC	PP
14.2.1.5	Mean (± SE) Plots of the Thermography Temperature Values Over Time	FAS
14.2.1.6	Mean (± SE) Plots of the Thermography Temperature Values Over Time	PP
14.2.2.1	Histogram of the Secondary Endpoint Thermography MAX	FAS
14.2.2.2	Scatter Plot of the Secondary Endpoint Thermography MAX	FAS
14.2.3.1	Histogram of the Secondary Endpoint Thermography DDD	FAS
14.2.3.2	Scatter Plot of the Secondary Endpoint Thermography DDD	FAS
14.2.4.1	Histogram of the Secondary Endpoint Thermography GRAD	FAS
14.2.4.2	Scatter Plot of the Secondary Endpoint Thermography GRAD	FAS

9. APPENDICES

9.1 SAS CODE

ANCOVA model

```
PROC MIXED data=test_proc;
  CLASS sequence subject period treatment;
  MODEL log_AUC = baseline sequence period treatment / ddfm=kenwardroger;
  RANDOM subject(seq) / subject=subject type=VC;
  LSMEANS trt /pdiff cl alpha=0.1;
run;
```

The above are sample codes and can be adapted to the study as needed.

9.2 SCHEDULE OF ASSESSMENTS

Table 1. Flow Chart for Trial Procedures

	Screening	Treatment	Treatment	End-of-Trial
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4***
Visit Window (days allowed from previous visit)		3–21 Days	3–7 Days	3–15 Days
Clinical assessments				
Informed consent	X			
In- & exclusion criteria	X			
Randomisation		X		
Withdrawal criteria		X	X	
Demographics	X			
Medical history & concomitant illness	X			
Weight, height and BMI	X			
Physical examination	X	X[1)]	X[1)]	X
Vital signs	X	X	X	X
Nailfold Capillaroscopy	X	X	X	
Thermal probe[2)]	X			
Modified Rodnan Skin Score	X			
Raynaud's Condition Score	X	X	X	
Thermography [3)]		X	X	

Cold challenge		X	X	
Concomitant medication	X	X	X	X
12-lead ECG	X			X
Holter ECG		X[4)]	X[4)]	
IMP administration [5)]		X	X[6)]	
Adverse events [7)]	X	X	X	X

* Visit 4: End-of-Trial Visit for all randomised subjects must be performed no later than 30 days after Visit 2 (randomisation). Visit 4 must also be performed if a randomised subject is withdrawn from the trial for any reason

1) Brief examination according to Investigator's judgement
2) Application of thermal probe for check of finger temperature not being below 27°C (exclusion criterion 10)
3) Please refer to Section 6.5.2 for details
4) Holter ECG from 30 mins prior to IMP administration and until 180 mins after
5) Withdrawal criteria must be checked prior to IMP administration (see Section 5.4)
6) Subjects will cross-over to opposite IMP (either C21 or placebo) compared to Visit 2
7) AEs will be reported from signing of informed consent until End of Trial participation (Visit 4)

Table 2. Laboratory Parameters

		Screening	Treatment	Treatment	End-of-Trial
		Visit 1	Visit 2	Visit 3	Visit 4*
Time tolerance from previous visit			3–21 Days	3–7 Days	3–15 Days
Laboratory Assessments					
Haematology[1)]		X			X
Biochemistry[2)]		X			X
Urinanalysis[3)]		X			X
HIV and Hepatitis B/C[4)]		X			
Thyroid Stimulating hormone (TSH)		X			
Plasma and whole blood samples for potential analyses of exploratory biomarkers		X	X[5)]	X[5)]	X
Plasma sample for potential analysis of exposure to C21			X[6)]	X[6)]	
Pregnancy tests (WOCBP)	beta-HCG for WOCBP. Follicular Stimulating Hormone (FSH), oestradiol for confirmation of WONCBP	X			X
	Urine dip-stick		X	X	

* Visit 4: End-of-Trial Visit for all randomised subjects.
  Visit 4 must be performed if a randomized subject is withdrawn from trial treatment for any reason

1) Haemoglobin (Hb), haematocrit (Erythrocyte volume fraction), platelet count (Trombocyte particle concentration [TPC]), leucocyte count, Mean Corpuscular Volume (MCV)
2) Albumin, alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase Gamma (ALP), bilirubin, Blood Urea Nitrogen (BUN), calcium, creatinine, C-Reactive Protein (CRP), Gamma Glutamyl Transferase (gGT), glucose, Lactate dehydrogenase (LD), potassium, sodium
3) PH, glucose, protein/albumin and Hb
4) HBsAg, HCVAb and HIV 1+ 2 AgAb
5) Samples to be obtained before IMP administration, before cold challenge, immediately after cold challenge, 15 min after cold challenge
6) Samples to be collected 15 min after cold challenge