Cover Page – SAP

Title of the trial: A randomized double-blind placebo-controlled phase 1 study on the safety, tolerability and pharmacokinetics/-dynamics of escalating single intravenous doses of AK1967 (Procizumab) in healthy male volunteers

NCT number: NCT06331884

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Attachments:

- SAP Version 1.0

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Clinical Statistical Analysis Plan CT-P1-001

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Latest Changes

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1	Initial version	09/02/2024

Older Changes

(from most new to oldest)

Version	**Change Description**	**Valid From** (mm/dd/yyyy)
Inapplicable	Inapplicable	Inapplicable

Attachments

- QMA_01049_Attachment_Clinical Statistical Analysis Plan CT-P1-001

Statistical Analysis Plan

Record Title: Clinical Statistical Analysis Plan CT-P1-001

Contents

1 Background and scope

This SAP is created for the study and protocol as stated below:

Table 1 Basic trial information.

EU-CT number	2023-507035-37-00
Trial name	Not applicable
Clinical Trial Protocol Version	Latest Version 5

All abbreviations are included in the Glossary

The following documents are attached to the QM record of this SAP:

- None (the Data Review Plan and Report are referenced in DHC VISION)

2 Study objectives

The objective of the AK1967 Phase 1 study is to evaluate the safety and tolerability of single escalating doses of AK1967 in healthy male volunteers. Main trial endpoints associated with this objective are fully described in the study protocol. To summarize, endpoints for this safety outcome are defined as differences between the different AK1967 dosage groups and the placebo group in:

- Reported number of adverse events after IMP administration
- Kinetics of vital signs at baseline and following IMP administration
- Local tolerability at site of IMP infusion
- Safety laboratory parameters at baseline and following IMP administration
- 12-lead electrocardiogram (ECG) parameters at baseline and following IMP administration

There are several secondary trial objectives. A full overview is again provided in the study protocol. To summarize, secondary trial measurements/endpoints discussed in this document include:

- Pharmacokinetics of the IMP (including AUC, C_{max}, Terminal $T_{1/2}$, clearance, VD)
- Pharmacodynamics (enzyme activity and concentration of DPP3) at baseline and following IMP administration.
- Pharmacodynamics related to the renin-angiotensin system (blood plasma levels of renin-angiotensin system metabolites) at baseline and following IMP administration
- Pharmacodynamics related to catecholamines (blood plasma levels of adrenergic metabolites) at baseline and following IMP administration
- Related to safety and pharmacodynamics: To determine the effects of AK1967 on inflammatory cytokines.

Primary objective(s):

- To assess the safety and tolerability of single escalating doses of AK1967 in healthy male volunteers.

Secondary objective(s):

- To determine the pharmacokinetics of single escalating doses of AK1967 in healthy male volunteers
- To determine the effects of AK1967 on DPP3 activity and concentration (pharmacodynamics) in healthy male volunteers
- To determine the effects of AK1967 on renin-angiotensin system metabolites (pharmacodynamics) in healthy male volunteers.
- To determine the effects of AK1967 on circulating adrenergic metabolite concentrations (pharmacodynamics) in healthy male volunteers.
- To determine the effects of AK1967 on inflammatory cytokines, including, but not limited to TNF, IL-6, IL-8, IL-10.

3 Trial design

Number of centers	1 center
Randomized?	Yes
Randomization ratio	Per dose group, 8 volunteers will participate of which 6 subjects will receive AK1967, and 2 will receive placebo. With 3 escalating dose groups, a total of 6 subjects will receive placebo. In total 24 subjects will participate resulting in a 1:1:1:1 ratio (treatment arm A: treatment arm B: treatment arm C: control group)
Blinded?	Double-blind
Dosing	Intravenous infusion (single i.v. dose) over 2 hours
Placebo controlled	Yes
Treatment arms	single i.v. dose of 3 mg/kg AK1967 (treatment arm A)
	single i.v. dose of 6 mg/kg AK1967 (treatment arm B)
	single i.v. dose of 12 mg/kg AK1967 (treatment arm C)
	single i.v. dose of Placebo (control group)
PK profile days	Pre-dose

	Day 1 (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24 hour ± 2 mins)
	Day 2 (± 1 hour) Day 3 (± 3 hours) Day 7 (± 1 day) Day 14 (± 2 days) Day 21 ((± 3 days) Day 28 (± 3 days)

Table 2 Treatment groups and sample sizes

Treatment	**N**
Treatment arm A: 3 mg/kg AK1967	6
Treatment arm B: 6 mg/kg AK1967	6
Treatment arm C: 12 mg/kg AK1967	6
Control group: Placebo	6
Total (randomization ratio per dose group 1:1:1:1)	24

For a flow chart of planned study procedures, please refer to the study protocol.

3.1 Sample size determination / power analysis

As the primary endpoint is safety and no comparative statistical analyses will be performed on this endpoint, a sample size calculation is not warranted. A generally accepted group size (for dose escalating phase 1 studies) of 6 subjects per dosing group will be included (in addition to the 2 placebo-treated subjects per dosing group, also resulting in a total of 6 in the placebo-group). A sample size of 6 subjects per treatment group is regarded as being large enough to justify continuation of the trial with the next dosing group in the absence of relevant adverse events related to the study medication. Likewise, one or more severe or serious adverse events considered to be related to the study medication could be enough to discontinue the study without a statistical difference in (S)AEs between the groups.

3.2 Hypotheses

The null hypothesis is that there is no difference between the AK1967 group (all doses combined) and the Placebo group regarding the primary safety endpoint.

4 Population

The main inclusion criteria are defined as:

- Written informed consent to participate in this trial prior to any study-mandated procedure.
- Male subjects aged 18 to 35 years inclusive.
- Subjects must agree to use a reliable way of contraception with their partners from study entry until one month after study drug administration.
- BMI between 18 and 30 kg/m², with a lower limit of body weight of 50 kg and an upper limit of 100 kg.
- Considered healthy as determined by medical history, physical examination, vital signs, 12-lead electrocardiogram, and conventional laboratory parameters.

Exclusion criteria are described in the protocol.

5 Endpoints for the primary, secondary objectives and exploratory endpoints

5.1 Endpoints for the primary objectives

Safety analysis:

The endpoints for the primary objective are to determine over the 28 days study period:

- Reported number of adverse events from baseline (just prior to the start of IMP administration) up until the last follow-up visit 28 days after IMP administration.
- (Changes in) vital signs from baseline to during the first 24 hours after start of IMP administration, as well as during the six follow-up visits. Vital signs included are: heart rate, blood pressure, peripheral oxygen saturation and body temperature.
- Local tolerability at site of i.v. infusion of the IMP.
- Safety laboratory parameters from baseline (just prior to the start of IMP administration) up until the last follow-up visit 28 days after IMP administration. Laboratory parameters included are: Hb, Htc, leukocytes, leukocyte differential blood count, thrombocytes, sodium, potassium, creatinine, urea, alkaline phosphatase, ALT, AST, bilirubin, GGT, CK, CRP, PT, APTT, fibrinogen.
- 12-lead electrocardiogram (ECG) at baseline (screening), compared to ECG's performed 2 hours, 9 hours and 7 days after start of IMP administration.

5.2 Endpoints for the secondary objectives

Secondary analysis:

- Pharmacokinetics of AK1967 (including AUC, C_{max}, Terminal $T_{1/2}$, Cl, V) from baseline (just prior to the start of IMP administration) up until the last follow-up visit 28 days after IMP administration.
- Pharmacodynamics (enzyme activity and concentration of DPP3) from baseline (just prior to start of IMP administration) up until the last follow-up visit 28 days after IMP administration.

- Pharmacodynamics related to the renin-angiotensin system (blood plasma levels of renin-angiotensin system responses) at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration.
- Pharmacodynamics related to catecholamines (blood plasma levels of adrenergic metabolites) at baseline (just prior to start of IMP administration) and at 2 and 10 hours after start of IMP administration.

Plasma levels of inflammatory mediators (including, but not limited to TNF, IL-6, IL-8, IL-10), measured from baseline (just prior to start of IMP administration) up until 24 hours after start of IMP administration.

Endpoints for Pharmacokinetics:

Key PK parameters, including:

- peak plasma concentrations (C_{max})
- time to C_{max} (t_{max})
- systemic exposure (AUC)
- volume of distribution (V)
- systemic clearance (Cl)
- terminal half-life ($t_{1/2}$)

5.3 Endpoints for the exploratory objectives

Not applicable

6 General conventions

6.1 Treatment groups

The following treatment groups will be considered for analysis where applicable and meaningful:

- AK1967 3 mg/kg
- AK1967 6 mg/kg
- AK1967 12 mg/kg
- Placebo

6.2 Layout of tables

For numbering of tables, listings and figures, please refer to section 9. Nevertheless, in order to facilitate programming, this numbering might be slightly changed in the final outputs.

Table 3 Layout of tables

Issue	Specification
Basic layout of end-of-text tables and listings (header, footnote)	Titles for all tables, figures, listings: • Study: CT-P01-001 / EU CT-2023-507035-37-00
Text (Title, footnotes, organizational variables and column headers, contents of table)	Case-sensitive, the first letter should be capitalized
Subject identification numbers	SUBJID (as recorded in eCRF)
Categorical Variables	Display of all possible categories (even if a category is not present in the data). In order to increase readability, large tables may also be presented with only the categories observed (e.g. frequency tables for adverse events by primary system organ class, preferred term and severity).
In case a category does not occur	Display absolute frequency as “0”.
Display of units	Presentation case-sensitive and in square brackets, e.g. Concentration [mg/mL].

6.3 General calculation rules

Data will be listed with the observed number of decimal places. Summary statistics will be presented to the same number of decimal places as the observed data, apart from the means and medians (to one more decimal place), and standard deviations (two more decimal places). Calculated data and summary statistics of calculated data will be presented with an appropriate number of decimal places.

In case of replicate measurements (e. g. three determinations of a parameter at one planned time-point), means of these measurements will be calculated and will be used for summary statistics without any prior rounding.

Data from unscheduled assessments will only be listed and not presented in summary statistics.

Relative study times and dates will be calculated with respect to the start time and date of the administration of study drug.

Calculation of relative days from onset of adverse events:

The relative day for onset of an adverse event is calculated as “day of onset” minus “day of start of treatment”.

Duration of adverse events:

The duration of an adverse event (expressed in days) is calculated as “stop date” minus “start date” plus 1.

If parts of the start/stop date of an adverse event are missing (day, month, or year), the relative day for onset and/or the duration of the adverse event are not calculated.

In order to determine if an adverse event or the worsening of an adverse event is a treatment-emergent event, the following rules will be used as a worst-case scenario:

- If the date (and time) of onset or change is completely known, the adverse event is considered as treatment-emergent, provided the day (and time) of onset or change is after or on the same day (and time) as first study treatment.
- If the time of onset or change is missing, but the day, month and year is known, the adverse event is considered as treatment-emergent, provided the day of onset or change is after or on the same day as first study treatment, unless other information (e.g. stop date/time) suggest otherwise.

Calculation of absolute changes from baseline:

- post-dose value - baseline value

Calculation of relative changes from baseline:

- (post-dose value - baseline value)/baseline value

Frequencies / percentages for qualitative data:

- Number of observations (frequency)
- Percent
  Percentages will be calculated as “frequencies” divided by the number of subjects in the population the analysis is based on (i.e. this may include missing data in the denominator).

6.4 Descriptive statistics

Basic descriptive statistics for continuous data comprise:

- Number of observations (N)
- Arithmetic mean
- Standard deviation (SD)
- Minimum (Min)
- Lower quartile (Q1)
- Median
- Upper quartile (Q3)
- Maximum (Max)

Descriptive statistics for continuous variables assumed as log-normally distributed (e. g. PK parameters):

- Number of observations (N)
- Number of observations ≥ LLOQ (NLOQ)
- Arithmetic mean
- Arithmetic SD
- Arithmetic coefficient of variation (CV)
- Geometric mean
- Geometric SD
- Geometric CV (%) (gCV)
- Q1
- Median
- Q3
- Min
- Max

7 Analysis sets and data review criteria

7.1 Data sets for analysis

A general definition of data sets for analysis will be:

Full analysis set

The full analysis set includes all randomized subjects who

- have received study medication
- have follow up data for the study primary and secondary outcomes

For the analysis per dose, subject that did not receive the dose per protocol should be grouped to the dosing group closest to the administered dose or analysed and reported separately.

Pharmacokinetic analysis set (PKS)

The pharmacokinetic analysis set includes all subjects who:

- received the study medication
- showed sufficient compliance concerning medication, i.e. received the full dose of study medication
- have sufficient concentration PK data to calculate reliable estimates of at least one PK parameter
- are without any protocol violation that would interfere with the interpretation of the PK data.

7.2 Handling of missing data, drop-outs, extreme values and protocol violations

For an overview of checks for identification of protocol violations and data review, please refer to the data review plan.

8 Data analysis

8.1 Statistical software

Statistical analysis will be performed using R statistical software, version 4.3.2 or higher (https://www.r-project.org/) on a Windows 7 (or higher) personal computer. The calculation of pharmacokinetic parameters will be performed with WinNonlin, version 8.4, Pharsight Corporation, Mountain View, CA.

For each script/test performed using R, the Rmarkdown function will be used to document the output of the computerized system for future reference.

8.2 Definitions, derived variables and handling of missing data

Definitions of time points / time windows

Baseline will be defined per parameter and will be the value/measurement at the following timepoints:

Parameter for	Timepoint
Demographics	Screening
Vital signs	Baseline (before IMP administration)
ECG	Baseline (Screening)
Local laboratory assessment	Baseline (before IMP administration)
Arterial blood gas analysis	Baseline (before IMP administration)

Parameter for	Timepoint
Biomarker (DPP3 concentration and activity)	Baseline (before IMP administration)

Derived variables

Extent of exposure:

Extent of exposure will be defined as total exposure in mg and calculated as:

$$total\ exposure\ [mg] = 20\ \frac{mg}{ml} * IMP\ volume\ [ml] * factor$$

where IMP volume is the body weight adjusted IMP volume before addition of NaCl and factor equals 1 for treatment group 12 mg/kg AK1967, 1/2 for treatment group 6 mg/kg AK1967, and 1/4 for treatment group 3 mg/kg AK1967. Total exposure will not be calculated for the Placebo treatment group.

In case of premature termination of the infusion, total exposure will additionally be multiplied by $\frac{volume\ administered\ [ml]}{50\ ml}$ in order to account for only partly administration of study drug.

Exposure per kg body weight will be calculated as:

$$Exposure\ per\ kg\ body\ weight\ \left[\frac{mg}{kg}\right] = \frac{total\ exposure\ [mg]}{body\ weight\ [kg]}$$

Body mass index (BMI):

$$BMI = \frac{Weight\ [kg]}{Height\ [m]^2}$$

Handling of missing values

Data from subjects who prematurely terminate the trial will be used to the maximum possible extent. Missing values will not be inputted.

8.3 Disposition

An overview of all subjects who entered the study, who were randomized and who completed the study will be provided by treatment group and overall.

8.4 Demographic data, screening and baseline characteristics

Demographic data and baseline characteristics will be summarized for the safety analysis set. Screening data (prior to study participation) will be shown as listings.

Demographic data and baseline characteristics

Summary statistics will be presented for age, weight, height, BMI, vital signs at screening and baseline (body temperature, heart rate, MAP, systolic and diastolic blood pressure, respiratory rate), Hb, Hct, leukocytes, leukocyte differential blood count, thrombocytes, sodium, potassium, creatinine, urea, alkaline phosphatase, ALT, AST, bilirubin, GGT, CK, CRP, PT, APTT, fibrinogen. Frequency tables for qualitative data (gender, age) will be provided. In case a value of one of these parameters is an outlier in a subject, this will be described separately.

In order to check for a potential disbalance of baseline characteristics of the three treatment groups, the Kruskal-Wallis test will be performed for continuous baseline characteristics.

Categorical variables will be compared using the Chi^2 test for contingency tables.

8.5 Analysis of primary endpoints

The analyses of the endpoints for safety and tolerability will be performed on the full analysis set.

Reported number of Adverse Events and local tolerability

Adverse events (including assessment of local tolerability at the infusion site, which is reported in an AE format as well) will be recorded from the moment of study drug infusion, throughout the study and the follow-up visits. The same accounts for serious adverse events. (S)AEs will be categorized into different organ-systems based on reported symptoms. (S)AEs represent a categorical variable, meaning they are summarized as counts and percentages for different dosage groups. Consequently, no formal data distribution assessment can be performed for this datatype.

An overview of all adverse events (original term) will be generated as subject data listings, including onset relative to start of treatment, duration, intensity, seriousness and potential relationship to the study drug, action taken and outcome. The incidence of treatment-emergent adverse events as well as number of subjects with treatment-emergent adverse events will be summarized by potential relationship to the study drug (certain, probable/likely, possible, unlikely, unrelated) for all treatment groups.

Vital signs

All determined vital signs (oxygenation, heart rate, systolic, diastolic -and mean arterial blood pressure) are registered continuously during the experimental protocol at a measurement frequency once per 5 seconds. Artefacts may be removed. This high-frequency data will first be averaged into 10-minute intervals (calculating simple moving averages), as a correction measure for registration of spurious measurements. The exception for this approach is temperature, for which no high-frequency measurements are performed, meaning no data transformation is required prior to statistical analysis. As vital signs measured during each of the follow-up visits also represent single

measurements, no data transformation of these measurements is required prior to statistical analysis. Minimum and maximum values for vital signs assessed within one day will be analyzed using descriptive summary statistics (including changes from baseline) by treatment group and time point (Day 1, Day 2, Day 3 etc.).

Data distribution for all vital signs (both averages of high-frequency data and single measurements) will be evaluated using the Kolmogorov-Smirnov test and, if applicable, normal/non-normal distribution will be reported. All variables will be expressed both as mean ± standard deviation (SD), as well as medians and interquartile ranges (IQR) in tables and graphical representations of the data. In case a value of one of these parameters is an outlier in a subject, this will be described separately.

Safety laboratory parameters & inflammatory parameters

All safety laboratory parameters (see the study protocol for a full overview) are measured at set intervals during the experimental protocol, as well as during each of the study's follow-up visits. All safety laboratory measurements represent single timepoint measurements, meaning no data transformation prior to analysis needs to be performed. Complete listings for laboratory values will be generated. Laboratory values outside the normal range will be marked. Minimum and maximum values will be reported (including changes from baseline) by treatment group and time point (Day 1, Day 2, Day 3 etc.). In case a value of one of these parameters is an outlier in a subject, this will be described separately.

Data distribution for all safety laboratory parameters will be evaluated using the Kolmogorov-Smirnov test and normal/non-normal distribution will be reported. All variables will be expressed both as mean ± standard deviation (SD), as well as medians and interquartile ranges (IQR) in tables and graphical representations of the data. In case a value of one of these parameters is an outlier in a subject, this will be described separately.

Results from laboratory tests will be summarized by treatment group and time point using descriptive summary statistics, including changes from baseline. Relative changes (for laboratory values) from baseline to follow-up visits (post-study drug administration minus baseline) will be categorized as:

- Maximum increase from baseline: (maximum value post-study drug administration – baseline) / baseline

  ≥ 1.5, ≥ 2.0

- Maximum decrease from baseline: (baseline - minimum value post-study drug administration) / baseline

  ≤ 0.5

A frequency table by treatment dose will be generated for these categorized changes.

In addition, changes from “from within normal range to above normal range” will be evaluated and categorized as:

- “changes to at least 1.5-time upper limit normal”, “changes to at least twice upper limit normal”.

A frequency table by treatment will be generated for these categorized changes from normal at baseline.

In case a value of one of these parameters is an outlier in a subject, this will be described separately.

12-lead ECG parameters

Non-quantifiable differences in ECG’s deemed to be of clinical relevance by a clinical investigator will be registered as an (S)AE. For differences in ECG-related (S)AEs, the same ground-rules apply as those listed under reported number of adverse events and local tolerability (please refer to this section). Frequencies of overall interpretation (normal, abnormal and clinically not significant, abnormal and clinically significant) will be tabulated by treatment group and time point.

For numerically quantifiable differences in ECG-parameters (e.g. QTc-time, see the study protocol for a full overview), data distribution will be assessed using the Kolmogorov-Smirnov test and normal/non-normal distribution will be reported. Individual measurement timepoints will be expressed as both mean ± standard deviation (SD), as well as medians and interquartile ranges (IQR).

Extent of exposure

Total exposure [mg] and relative exposure [mg/kg] will be analyzed by descriptive summary statistics.

8.6 Analysis of secondary endpoints

Pharmacokinetic data analyses

The PK parameters will be analyzed by descriptive summary statistics. AK1967 concentration-time profiles for individual subjects will be analyzed with non-compartmental approaches using a validated commercially available software package (WinNonlin, version 8.4, Pharsight Corporation, Mountain View, CA). Nominal times will be used in the analysis. For data reported as BLQ and ALQ (below and above limit of quantification, respectively), the low and high limit of detection value will be used in the analysis.

Pharmacokinetic (PK) parameters will be calculated including area under the concentration–time curve (AUC), maximum concentration (C_{max}), time to C_{max} (Tmax), and terminal half-life (t ½).

The maximum drug concentration (C_{max}) and time to maximum drug concentration (T_{max}) will be determined from observed values. The area under the curve (AUC_{0-t}) will be calculated for PCZ plasma concentration-time profiles using the trapezoidal method derived from non-compartmental analysis from time zero (i.e. start of drug infusion) to the last detectable concentration.

Data distribution will be assessed using the Kolmogorov-Smirnov test and normal/non-normal distribution will be reported. Data will be displayed both tabular and graphically as means ± SD, as well as medians ± IQR. In case a value of one of these parameters is an outlier in a subject, this will be described separately.

Dose proportionality of C_{max} and $AUC_{0-\infty}$ of the different AK1967 dose groups will be calculated by dividing the specific PK parameter by the corresponding value in the lower dose groups and comparing with the corresponding fold change in dose. This is to assess which dosage groups should be reported as being non-dose proportional.

Pharmacokinetic parameters

The following parameters will be determined from the individual plasma concentration-time profiles of AK1967 using non-compartmental methods based on actual relative sampling times:

Single concentration data will be shown with the relevant number of decimal places as provided by the analytical lab. Derived parameters, as far as they do not present single concentrations such as C_{max}, will be listed with an appropriate number of relevant digits (at least 3).

Exposure parameters (C_{max} and AUC_{0-last}) will also be dose-normalized (i.e. divided by dose) for the determination of dose-dependency.

Descriptive pharmacokinetic analyses

The PK analysis will be performed for all active treatment groups.

The individual time courses of the plasma concentrations of AK1967 will be listed and summarized descriptively by treatment group and time point.

Summary statistics by time point will be calculated including only those samples which were taken according to the pre-defined sampling schedule, i.e.: Day 1 (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24 hour ± 2 mins), Day 2 (± 1 hour), Day 3 (± 3 hours), Day 7 (± 1 day), Day 14 (± 2 days), Day 21 ((± 3 days), and Day 28 (± 3 days).

For the calculation of summary statistics, when data is reported as BLQ and ALQ (below and above limit of quantification, respectively), the low and high limit of detection value will be used in the analysis to obtain the means, standard of deviations, coefficient of variation, medians and interquartile ranges.

Individual plasma concentration-time profiles will be plotted for AK1967 (up to 6 profiles in one plot) for each dose group considering all subjects with available PK concentration data using actual relative sampling times. Mean (SD), as well as median (IQR) of AK1967 plasma concentrations will be plotted for all active treatment groups. Individual and geometric mean concentration versus time curves will be plotted using both linear and semi-logarithmic scaling.

Summary statistics will be calculated for each pharmacokinetic parameter by dose group based on PKs.

Pharmacodynamic data analyses (effects of AK1967 on DPP3 enzyme activity, concentration, renin-angiotensin and adrenergic system metabolites)

- To determine the effects of AK1967 on DPP3 activity and concentration in healthy male volunteers.
- To determine the effects of AK1967 on renin-angiotensin system metabolites in healthy male volunteers.
- To determine the effects of AK1967 on circulating adrenergic metabolite concentrations in healthy male volunteers.
- To determine the effects of AK1967 on inflammatory cytokines, including, but not limited to TNF, IL-6, IL-8, IL-10.

Different biomarkers/measurements associated with AK1967's pharmacodynamic activity (see the study protocol for a full overview) are measured at set intervals during the experimental protocol. DPP3 enzyme activity and concentrations measurements are also performed during each of the study's follow-up visits. All pharmacodynamic biomarker measurements represent single timepoint measurements, meaning no data transformation prior to analysis needs to be performed. DPP3 enzyme activity, concentration, adrenergic and renin-angiotensin system metabolite results will be analysed by descriptive summary statistics for the different scheduled determination time points as well as expressed as changes from baseline.

Data distribution for all pharmacodynamic biomarkers will be evaluated using the Kolmogorov-Smirnov test and normal/non-normal distribution will be reported. All variables will be expressed both as mean ± standard deviation (SD), as well as medians and interquartile ranges (IQR) in tables and graphical representations of the data. In case a value of one of these parameters is an outlier in a subject, this will be described separately. Between group differences in pharmacodynamic biomarkers will be assessed using one-way-RM-ANOVA tests (for normally distributed data), or Kruskal-Wallis tests (for non-normally distributed data). In case of a p-value of <0.05 for these tests (implying the presence of dosage groups with differences in a vital sign at a specific timepoint), subsequent Bonferroni or Mann-Whitney-U post-hoc tests will be performed (depending on data distribution), to assess which subgroups are different, and in what direction.

8.7 Additional analyses

Pharmacodynamic data analyses (effects of AK1967 on DPP3 enzyme activity, concentration and renin-angiotensin metabolites)

- To determine the effects of AK1967 on DPP3 concentration (corrected and non-corrected) in healthy male volunteers.
- To determine the correlation between DPP3 concentration (corrected and non-corrected) and DPP3 activity.
- To determine the effects of AK1967 on renin-angiotensin system response measured in non-stabilized (equilibrium) plasma samples at day 7.
- To determine the effects of AK1967 on renin-angiotensin system metabolites measured in stabilized (protease inhibitor) and non-stabilized (equilibrium) plasma samples over all timepoints.
- To determine the correlation between renin-angiotensin system modulation measured in stabilized (protease inhibitor) and non-stabilized (equilibrium) plasma samples over all timepoints.
- To determine the correlation between DPP3 activity and renin-angiotensin modulation measured in stabilized (protease inhibitor) and non-stabilized (equilibrium) plasma samples.
- To determine the correlation between DPP3 concentration (corrected and non-corrected) and renin-angiotensin modulation measured in stabilized (protease inhibitor) and non-stabilized (equilibrium) plasma samples.

All pharmacodynamic biomarker measurements represent single timepoint measurements, meaning no data transformation prior to analysis needs to be performed. DPP3 enzyme activity, concentration (corrected and non-corrected values) and renin-angiotensin system metabolite (in stabilized and non-stabilized plasma) results will be analysed by descriptive summary statistics for the different scheduled determination time points, as well as expressed as changes from baseline. Correlations between pharmacodynamic parameters will be reported.

Data distribution for all pharmacodynamic biomarkers will be evaluated using the Kolmogorov-Smirnov test and normal/non-normal distribution will be reported. All variables will be expressed both as mean ± standard deviation (SD), as well as medians and interquartile ranges (IQR) in tables and graphical representations of the data. In case a value of one of these parameters is an outlier in a subject, this will be described separately. Between group differences in pharmacodynamic biomarkers will be assessed using one-way-RM-ANOVA tests (for normally distributed data), or Kruskal-Wallis tests (for non-normally distributed data). In case of a p-value of <0.05 for these tests (implying the presence of dosage groups with differences in a vital sign at a specific timepoint), subsequent Bonferroni or Mann-Whitney-U post-hoc tests will be performed (depending on data distribution), to assess which subgroups are different, and in what direction.

Additional analyses related to the effects of AK1967 on safety endpoints

Between group differences in vital signs will be assessed using one-way-RM-ANOVA tests (for normally distributed data), or Kruskal-Wallis tests (for non-normally distributed data). In case of a p-value of <0.05 for these tests (implying the presence of dosage groups with differences in a vital sign at a specific timepoint, subsequent Bonferroni or Mann-Whitney-U post-hoc tests will be performed (depending on data distribution), to assess which subgroups are different, and in what direction.

Between group differences over time for vital signs, safety laboratory and inflammatory parameters (i.e. laboratory/inflammatory parameter changes during the course of the experimental protocol) will be analyzed using two-way-RM-ANOVA tests (for normally distributed data), or two-way-RM-ANOVA tests on ranked transformed data (for non-normally distributed data). Both tests will report the time*group interaction term to establish significance, which will be defined as p <0.05.

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