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Protocol: **NKF-INS(G)-101**
NCT No.: **06627478**
Subject: **Cover Page for Statistical Analysis Plan (SAP)**

SAP V2.0 29JUL2025 for NKF-INS(G)-101 is attached with NCT06627478.

scigenix

STATISTICAL ANALYSIS PLAN

Protocol title: A single center, single-dose, double-blind, randomized, three-period, three-treatment, six-sequence, crossover study to demonstrate pharmacokinetic and pharmacodynamic similarity between NKF-INS(G), US-Lantus®, and EU-Lantus® using the euglycemic clamp technique in healthy male adult volunteers.	
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Glossary of abbreviations

Abbreviation	Description
ADaM	Analysis Data Model
ADPP	ADaM Pharmacokinetic Parameter dataset
AE	Adverse event
ADL	Activities of daily living
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
Anti-HBc	Antibody to hepatitis B core antigen
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{0-24}	AUC from 0 h to 24 h
AUC_{0-6h}	AUC from 0 h to 6 h
AUC_{6-12h}	AUC from 6 h to 12 h
AUC_{0-12h}	AUC from 0 h to 12 h
AUC_{12-18h}	AUC from 12 h to 18 h
AUC_{18-24h}	AUC from 18 h to 24 h
AUC_{12-24h}	AUC from 12 h to 24 h
$AUC_{0-\infty}$	AUC from 0 h to the last quantifiable concentration-time extrapolated to infinity
BLQ	Below the limit of quantification
BMI	Body mass index
CFB	Change from baseline
CI	Confidence interval
CK	Creatine kinase
CL/F	Total body Clearance
C_{max}	Maximum observed plasma exogenous insulin glargine concentration
COVID-19	Coronavirus disease 2019
CRU	Clinical research unit
CSP	Clinical study protocol
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
ECG	Electrocardiogram

EDTA	Ethylenediaminetetraacetic acid
eCRF	Electronic Case Report Form
EOS	End of study
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GIR	Glucose infusion rate
GIRAUC	Area under the GIR-time curve (calculated as the exact area under the stepwise constant function)
$GIRAUC_{0\text{-}24h}$	GIRAUC from 0 h to 24 h
$GIRAUC_{0\text{-}6h}$	GIRAUC from 0 h to 6 h
$GIRAUC_{6\text{-}12h}$	GIRAUC from 6 h to 12 h
$GIRAUC_{0\text{-}12h}$	GIRAUC from 0 h to 12 h
$GIRAUC_{12\text{-}18h}$	GIRAUC from 12 h to 18 h
$GIRAUC_{18\text{-}24h}$	GIRAUC from 18 h to 24 h
$GIRAUC_{12\text{-}24h}$	GIRAUC from 12 h to 24 h
$GIRAUC_{0\text{-}\infty}$	GIRAUC from 0 h to the last quantifiable concentration-time with extrapolation to infinity
$GIRAUC_{0\text{-end of clamp}}$	GIRAUC from 0 h to end of clamp
GIR_{max}	Maximum GIR
G_{tot}	Total amount of glucose infused during clamp procedure
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
HCT	Hematocrit
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICF	Informed Consent Form
ICH	International Council for Harmonisation
INR	International normalized ratio
IQ	Installation Qualification
IU	International Units
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LDH	Lactate dehydrogenase
M1	Metabolite 21A-Gly-human insulin
M2	Metabolite 21A-Gly-des-30B-Thr-human insulin
MARD	Mean Absolute Relative Difference
MedDRA	Medical Dictionary for Regulatory Activities

MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRT	Mean residence time
NCA	Noncompartmental analysis
NCI	National Cancer Institute
OQ	Operational Qualification
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Pharmacokinetic parameters
PQ	Performance Qualification
PT	Prothrombin time
QTcF	QT-interval corrected by Fridericia's formula
RBC	Red blood cell
SAE	Serious adverse event
SAF	Safety analysis set
SAP	Statistical Analysis Plan
SC	Subcutaneous(ly)
SD	Standard deviation
SOC	System organ class
SOP	Standard Operating Procedure
SST	Serum separator tube
$t^1/_2z$	Apparent terminal elimination half life
TEAE	Treatment emergent adverse event
$TGIR_{max}$	Time to maximum GIR
TGV	Target Glucose Values
TLF	Tables, listings and figures
T_{max}	Time to maximum plasma exogenous insulin glargine concentration
$TGIR_{max}$	Time to maximum GIR; US: United States
US	United States
V/F	Volume of distribution
WBC	White blood cell
λz	Apparent terminal elimination rate constant

1. Overview

1.1 Introduction

This document describes the rules and conventions to be used in the presentation and analysis of the Xentria, Inc. study titled “A single center, single-dose, double-blind, randomized, three-period, three-treatment, six-sequence, crossover study to demonstrate pharmacokinetic and pharmacodynamic similarity between NKF-INS(G), US-Lantus®, and EU-Lantus® using the euglycemic clamp technique in healthy male adult volunteers”.

This statistical analysis plan (SAP) is based on protocol NKF-INS(G)-101, Version 3.0, dated 29-Aug-24. This SAP describes all preplanned analyses as specified in the protocol, adding clarification and details where appropriate. If circumstances should arise during the study rendering the analysis inappropriate, or if in the meantime improved methods of analysis should come to light, different analyses may be made. Any deviations from the statistical methodology, reasons for such deviations and all alternative or additional statistical analyses that may be performed, will be described in the clinical study report (CSR).

This will be a phase 1, double-blind, randomized, three period crossover study to demonstrate pharmacokinetic (PK) and pharmacodynamic (PD) similarity between NKF-INS(G), US-Lantus®, and EU-Lantus®, as well as to assess the safety of NKF-INS(G), using the euglycemic clamp technique in healthy male adult volunteers at a single study center.

2. Study objectives and endpoints

Objectives	Endpoints
Primary	
• To compare the PK of NKF-INS(G) to US-approved and EU-authorized Lantus to demonstrate PK similarity for metabolite 21A-Gly-human insulin (M1).	• $AUC_{0\text{-}24h}$ of M1.
Secondary	
• To evaluate additional PK parameters of NKF-INS(G), US-approved and EU-authorized Lantus to demonstrate similarity for insulin glargine and/or M1.	• PK parameters for plasma insulin glargine and/or M1 concentrations: Cmax, $AUC_{0\text{-}24h}$, $AUC_{0\text{-}6h}$, $AUC_{6\text{-}12h}$, $AUC_{0\text{-}12h}$, $AUC_{12\text{-}18h}$, $AUC_{18\text{-}24h}$, $AUC_{12\text{-}24h}$ (fractional area under the plasma concentration-time curve), Tmax, $AUC_{0\text{-}\infty}$.
• To compare the PD of NKF-INS(G) to US-approved and EU-authorized Lantus by examining glucose	• PD parameters: $GIRAUC_{0\text{-}24h}$, $GIRAUC_{0\text{-end of clamp}}$, $GIRAUC_{0\text{-}\infty}$, $GIRAUC_{0\text{-}6h}$, $GIRAUC_{6\text{-}12h}$,

infusion rate (GIR) profiles after a single sub-cutaneous (SC) dose.	$GIRAUC_{0\text{-}12h}$, $GIRAUC_{12\text{-}18h}$, $GIRAUC_{18\text{-}24h}$, and $GIRAUC_{12\text{-}24h}$ (fractional area under the GIR-time curve), GIR_{max}, $TGIR_{max}$, and $G_{tot.}$
• To assess the safety of NKF-INS(G).	• Adverse Event (AE) assessments (including injection site reactions), clinical laboratory investigations (hematology, clinical chemistry [including glucose], coagulation, and urinalysis), vital signs, physical examinations, 12-lead electrocardiogram (ECG), prior and concomitant medication assessments.
Exploratory	
• To assess endogenous insulin suppression via C-peptide measurements.	• Uncorrected C-peptide concentrations.
• To compare the PK of NKF-INS(G) to the US-approved and EU-authorized Lantus to demonstrate PK similarity for metabolite 21A-Gly-des-30B-Thr-human insulin (M2) if sufficient concentration-data are available.	• PK similarity for M2: $AUC_{0\text{-}24h}$, C_{max}, and $AUC_{0\text{-}\infty}$ of M2.
• To explore evaluation of additional PK parameters of NKF-INS(G), US-Lantus, EU-Lantus for insulin glargine, M1, and/or M2, as appropriate based on concentration-time data where feasible.	• If possible, i.e., if the terminal phase of plasma concentration-time profile has sufficient data points, apparent terminal elimination half-life ($t^1/_2z$), apparent terminal elimination rate constant (λz), mean residence time (MRT), full body clearance (CL/F), and volume of distribution (V/F) will be calculated.
• Measurement of assay sensitivity of plasma concentrations using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) data.	• Comparative analysis of LC-MS/MS plasma concentration profiles.

The exploratory objective of the measurement of assay sensitivity of plasma concentrations using LC-MS/MS data through comparative analysis of LC-MS/MS plasma concentration profiles is not part of the

analyses under this SAP and will be described in separate study documents.

3. Study design

3.1 Design overview

Study NKF-INS(G)-101 is a Phase 1, 24-hour euglycemic glucose clamp study conducted using a randomized, double-blind, three-period, three-treatment, six-sequence crossover design. The study will compare single doses of the proposed insulin biosimilar (NKF-INS(G)) with respective EU- and US- reference products in healthy male participants.

Written informed consent will be obtained from all participants, and the study will be conducted in accordance with the principles of Good Clinical Practice (GCP) as defined by the International Council for Harmonisation (ICH).

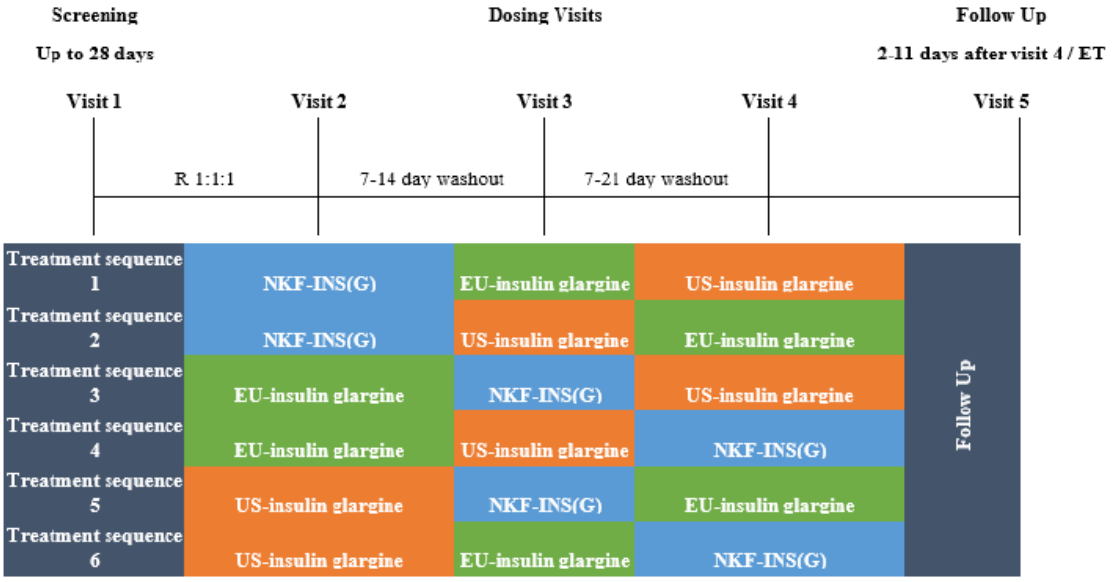
The study will consist of:

- a screening visit (Days -28 to -1),
- three treatment periods each consisting of 1-day dose administrations and euglycemic clamp periods (periods 1, 2, and 3) separated by a washout period of 7-14 calendar days between treatment periods 1 and 2, and a washout period of 7-21 calendar days between treatment periods 2 and 3, and
- a follow-up visit 2-11 days after the last dose.

The entire study duration will be a maximum of 11 weeks.

Three insulin products (NKF-INS(G), EU-Lantus®, and US-Lantus®) will be administered over three treatment periods. Participants will be admitted to the clinical research unit (CRU) on Day -1. Eligible participants will be randomized on Day 1 to one of six treatment sequences in a 1:1:1:1:1:1 ratio, receiving a single SC dose of 0.5 IU/kg administration of one of the three study drugs on each dosing day.

Figure 1: Study Design (from protocol v3.0, 29-Aug-2024)



Abbreviations: ET = Early Termination; EU = European Union; US = United States

A 24-hour euglycemic glucose clamp will be conducted using a manual clamp technique, which includes monitoring the participant's blood glucose and adjusting the glucose infusion rate (GIR) to maintain blood glucose close to the target blood glucose concentration. The clamp procedure will be initiated, maintained, and terminated, in accordance with the site standard operating procedures (SOPs). The participant's blood glucose will be maintained within a pre-defined target window per site SOP and clinical judgment for the participant by manually adjusting the GIR.

Blood samples will be collected at pre-specified intervals before and up to 24 hours after dosing, for measurement of blood glucose, plasma insulin, and serum C-peptide.

The following steps will be taken to minimize variability:

- Prior to each dosing, participants will fast for 10-12 hours
- Individual participants will be dosed at about the same time of day within each treatment
- The injection site and technique will be standardized between treatment periods
- Any occurrence of a drop in glucose below 3.3 mmol/L will be recorded as this may induce counter-regulatory hormones

3.1.1 Sample Size Considerations

A literature review of similar studies was conducted in order to inform the assumptions for the sample size, for the primary endpoint of $AUC_{0\text{-}24h}$ of M1.

The Coefficient of Variation (CV%) value ranged from 14.7% to 33.1% for the primary endpoint of $AUC_{0\text{-}24h}$ of M1. The CV% value

ranged from 17.46% and 42.91% for the secondary PK and PD endpoints.[2-5]

Based on the above data, a CV% of 43% was selected.

The sample size was calculated using a modification of Zhang's method [1] in R software, R Version 4.4.1 (see Section 5.5 of the SAP) utilizing the following:

- Alpha: 2.5%
- Target power: 80%
- Bioequivalence margins: 80%, 125%
- True ratio: 95%
- CV%: 43

Considering a 95% confidence interval (CI) and 15% drop out rate, 114 participants are planned to be enrolled to have at least 96 evaluable participants. In order to ensure a balanced number of evaluable participants per treatment sequence, 16 participants per sequence are required for 96 evaluable participants. Considering the drop out rate, randomization has been set-up as 19 participants per treatment sequence.

3.2 Schedule of events

Refer to *Table 3 in the clinical study protocol (CSP) for NKF-INS(G)-101 v3.0 29-Aug-2024*.

Table 3.2: Pharmacokinetic, pharmacodynamic, C-peptide and glucose sampling schedule (from protocol v3.0, 29-Aug-2024)

	Pre-dose (minutes)					Post-dose (hours)												
Sample	60	30	15	5	0	0.5	1	2	3	4	6	8	9	12	15	18	21	24
Insulin PK, (plasma) (including M1/M2)	X				X	X	X	X	X	X	X		X	X	X	X	X	X
C-peptide (serum)	X				X	X	X	X	X	X	X		X	X	X	X	X	X
Blood glucose	X	X	X	X	X	Every 5 minutes →							Every 10 minutes →					

4. Analysis sets

Agreement and authorization of participants included/excluded from each analysis set will be reached prior to final database hard lock. Review of participant inclusion/exclusion from analysis sets will be done in a blinded fashion. The Sponsor will supply a list of all participants to be excluded from the relevant analysis sets, including the reason(s) for exclusion from the analysis sets.

All protocol deviations (major or minor) that occur during the study will be considered for their severity/impact in terms of adversely influencing the integrity or reliability of results and may be taken into consideration when participants are assigned to analysis sets. All analysis exclusions will be clearly documented with reasons for exclusion as well as detailed in the CSR.

The PK and PD parameter analysis sets may include participants that are excluded from certain parameter summary statistics and bioequivalence analysis. Exclusion of certain PK and PD parameters will be based on the reliability of the parameter used in the calculation of summary statistics. Please see Section 18 and 19 of the

SAP for more information regarding the assessment of reliability of PK and PD parameters estimated using Non-Compartmental Analysis (NCA).

4.1 Screening Analysis Set

This analysis set will include all participants who provided written informed consent, and provided demographic and/or baseline screening assessments, regardless of the participant's randomization status. Screening failures will be included in this analysis set. This analysis set will be used for the analyses of disposition.

4.2 Randomized Analysis Set (RAS)

This analysis set includes all individuals who signed the Informed Consent Form (ICF) and were randomized. The RAS will be used primarily for listings, unless indicated otherwise. Participants in the RAS will be analyzed under the treatment as randomized.

4.3 Safety Analysis Set (SAF)

This analysis set will include all participants who received any amount of study treatment. Analyses based on the SAF will reflect the actual study treatment administered to participants, regardless of the randomization list. This analysis set will be used for the analysis of safety.

4.4 Pharmacokinetic Concentration Analysis Set (PKCS)

The PKCS will include all participants in the SAF who additionally have at least 1 post-baseline reportable PK result without any major protocol deviations affecting PK. The PKCS will be used for the analyses of PK concentration data. Participants in the PKCS will be analyzed under the treatment as actually received.

4.5 Pharmacokinetic Parameter Analysis Set (PKPS)

The PKPS will include all participants in the PKCS who have at least 1 PK parameter derived without any major protocol deviations that would affect the evaluability of the pharmacokinetic profile. The PKPS will be used for the analyses of PK parameters. Participants in the PKPS will be analyzed under the treatment as actually received.

4.6 Pharmacodynamic Parameter Analysis Set (PDPS)

The PDPS will include all participants in the SAF who have at least 1 PD parameter derived without any major protocol deviations that would affect the evaluability of the pharmacodynamic profile. The PDPS will be used for the analyses of PD parameters. Participants in the PDPS will be analyzed under the treatment as actually received.

5. General considerations

5.1 Visit and date conventions

Visit day will be calculated from the reference start date which will be used to present start/stop day of assessments and events. The *reference start date* is defined as the date of first study treatment administration. The following conventions will be used for visit references:

- $Visit\ day = date\ of\ event - reference\ start\ date + 1$
- $Visit\ week = \frac{visit\ day}{7},\ rounding\ up\ to\ next\ whole\ number$
- $Visit\ month = \frac{visit\ day}{30.44},\ rounding\ up\ to\ next\ whole\ number$

No visit windowing (i.e. remapping of visits based on visit windows) will be performed for this study. The assigned nominal visit will be used for by-visit summaries. In the situation where the assessment/event date is partial or missing, visit day/week/month, and any corresponding durations will appear missing in the data listings. Unscheduled measurements will not be included in by-visit summaries. In the case of a retest (same visit number assigned), the last available measurement for that visit will be presented for by-visit summaries. Data listings will include scheduled, unscheduled, retest and early discontinuation data. Study visit will be assigned as delineated in Table 5.1:

Table 5.1: Study Visit Numbering

	Screen	**Euglycemic Clamp Procedure Treatment Periods 1-3**				**Post-Study Visit**
		Admission		Clamp Procedure [1]	Post Clamp	Within 2-11 Days After Last Administration of Study Drug
Visit Day	-28 to -1	Period 1	-1	1	2	23 – 53[2]
		Period 2	9 - 16	10 - 17	11 - 18	
		Period 3	19 - 40	20 - 41	21 - 42	
Visit No.	1	Period 1	2	3	4	11
		Period 2	5	6	7	
		Period 3	8	9	10	

[1] Randomization and final eligibility check will only be done in treatment period 1
[2] There will be a washout period of 7-14 calendar days between treatment period 1 and 2, and a washout period of 7-21 calendar days between treatment periods 2 and 3

5.2 Baseline

Unless stated otherwise, baseline is defined as the last non-missing observation made prior to the first administration of study treatment.

5.3 Statistical tests

For the purpose of the analyses described in this SAP and to align study objectives per protocol with Regulatory Authority guidance documents, the terms “biosimilarity/biosimilar” and “bioequivalence” are used interchangeably.

Bioequivalence is assumed if the two-sided 90% CI (PK and US-PD) / two-sided 95% CI (EU-PD) of the test and reference ratio of the PK/PD parameters fall within the limits of 80.00%-125.00%. For other PK and PD parameters, the same assessment of ratio and EQ margin will be performed, but confirmation of bioequivalence will be based on the primary PK endpoint.

5.4 Common calculations

For quantitative measurements, change from baseline (CFB) will be calculated as: (Test value at Visit Day X – Baseline value), where the baseline value is defined as the last non-missing observation assessed prior to first exposure to study treatment.

5.5 Software

All analyses will be performed using R Version 4.4.1 or higher. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.[6]

The validation of R used for this study was conducted in accordance with the Computerized System GxP Categorization and Overall Risk Assessment, which defined the system's validation scope. Testing was performed in alignment with user requirements and system functionality and documented using applicable SOPs.

The validation process included three key facets: **Installation Qualification (IQ)**, which ensured proper software installation on the target hardware and operating system, validated the configuration and setup, and addressed any installation issues; **Operational Qualification (OQ)**, which involved executing test scripts to verify system functionality, data integrity, performance, scalability, and interactions with other systems; and **Performance Qualification (PQ)**, which further evaluated system usability, workflow, and overall functionality. All validation activities were successfully completed, confirming that the computerised system used for conducting the statistical analysis operated as intended and met the specified requirements.

R CMD checks are run to ensure the package is developed according to a high level of standards by the Comprehensive R Archive Network (CRAN). This automatically checks for problems that may have been difficult to detect during development, and forms an integral part of the validation of the code base by producing the necessary documented evidence which provides a high degree of assurance that the code base consistently produces a product meeting its expected results and quality attributes.

6. Statistical considerations

6.1 Missing data

The following missing safety data will be imputed for this study:

- partial prior/concomitant medication start and/or stop dates, which will be imputed as described in Section 13.1 of the SAP;

- partial AE start and/or stop dates, missing AE severity and/or missing AE relationship to study treatment, which will be imputed as described in Section 14.1 of the SAP.

All other incomplete safety and missing PK data, including dates and times, are treated as such.

7. Output presentations

Summary tables will be presented stratified by treatment sequence as randomized to, unless otherwise specified.

The templates provided in the separate output templates document describe the format and content for presentation of tables, listings and figures (TLFs).

Categorical variables will be summarized and presented with the following nomenclature: n = frequency and % = percentage. Categories of NOT REPORTED, MISSING, and/or UNKNOWN may be excluded from summaries if null (0).

For continuous variables, data will be summarized with the number of participants (N), arithmetic mean, standard deviation (SD), minimum, median and maximum.

All percentages (%) for a specific summary are calculated using the total number of participants included in the relevant analysis set as the denominator, unless otherwise specified. Tables will summarize relevant data from the electronic case report form (CRF).

Data listings will be based on all participants randomized, unless otherwise specified.

8. Participant disposition and withdrawal

8.1 Variables and derivations

Participant end of study classifications are defined as follows:

8.1.1 Screening failure

Participants for whom informed consent was obtained and documented in writing (i.e. the participant signed an ICF), but who were not randomized to study treatment. Individuals who do not meet the criteria for participation in this study may be rescreened only once, at the discretion of the Investigator.

8.1.2 Completed treatment

A participant will have fulfilled the requirements for treatment completion if/when the participant has completed all three treatment periods.

8.1.3 Early withdrawal

Participants may withdraw from the study at any time with or without reason. The Investigator also has the right to discontinue or terminate participants from the study in the event of concomitant disease, AEs, or any reason that would benefit the participant. If a participant withdraws from the study for any reason, the study site must immediately notify the clinical monitor. The date and reason for study discontinuation must be recorded in the eCRF. Participants

who discontinue early, irrespective of the reason for discontinuation from the study, will be asked to complete the End of Study (EOS) assessments at the time of the participant's withdrawal, as outlined in the schedule of assessments (*Table 3 in the CSP for NKF-INS(G)-101*).

8.1.4 Completed study

A participant will have fulfilled the requirements for study completion if/when the participant has completed all three treatment periods, and the EOS visit after last administration of the study treatment.

8.1.5 Lost to follow-up

The participant is considered lost to follow-up if the participant stopped coming for visits and study personnel were unable to contact the participant. In cases in which the participant is deemed lost to follow up, prior to this designation, the Investigator (or designee) must make every effort to contact the participant (e.g., telephone calls, certified letter to the participant's last known mailing address, or local equivalent methods, and efforts to reach the participant's emergency contact). The site will make 3 attempts to regain contact with the participant. These contact attempts must be documented in the participant's records.

8.2 Analysis

Participant disposition and withdrawals will be summarized and presented in data listings for the Screening Analysis Set.

The number of participants included in the relevant analysis sets, as well as the number of participants excluded with reasons for exclusion from the relevant analysis sets (Section 4 of the SAP) and protocol violations/deviations will be summarized and listed in data listings for the RAS.

9. Participant demographics and other baseline characteristics

9.1 Variables and derivations

The following demographic and other baseline characteristics will be summarized by treatment sequence:

- Age (Years) (Calculated relative to date of informed consent, as provided by the participant and recorded in the eCRF)
- Sex
- Race
- Ethnicity
- Height (cm) at Screening
- Weight (kg) at Screening
- BMI (as collected in the eCRF)

The following additional demographic and baseline characteristics will be listed:

- The date of written informed consent
- Year of birth

9.2 Analysis

Demographic data and other baseline characteristics will be summarized by treatment sequence as randomized to for the RAS and presented in data listings for the Screening Analysis Set.

10. Medical and treatment history

10.1 Variables and derivations

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). See the relevant Data Management Plan for details.

Medical history will be recorded at screening. The recorded medical history will be updated, if necessary, at admission to treatment period 1. Investigators should document the occurrence, signs, and symptoms of the participant's preexisting conditions, including all prior significant illnesses. Medical history will include alcohol consumption and smoking history, if applicable.

Illnesses first occurring or detected during the study and/or worsening of a preexisting illness during the study are to be documented as AEs in the eCRF in accordance with Section 12.6 in the CSP of NKF-INS(G)-101 v3.0 29-Aug-2024. All changes that are not present at baseline or described in the medical history and identified as clinically noteworthy must be recorded as AEs.

10.2 Analysis

Medical history will be summarised by system organ class (SOC) and preferred term for the RAS. It will also be listed for each participant by SOC and preferred term, for the RAS.

11. Exposure to study treatment

11.1 Variables and derivations

The date of first study treatment administration will be derived as the first date of dosing from the "IP administration" eCRF page. As this is a single dose study, the date of the first study treatment administration of a particular study treatment will also be the last date of the particular study treatment's administration.

11.2 Analysis

Exposure to each study treatment administered will be presented in data listings for the SAF.

12. Study treatment compliance

12.1 Variables and derivations

Administration of the study treatment will be supervised by unblinded study site personnel to ensure compliance. An unblinded monitor will periodically review the study records to ensure compliance.

12.2 Analysis

Overall compliance to study treatment will be summarized for each study treatment for the SAF and presented in data listings. Dosing administration errors will be listed for the same Analysis Set, citing the treatment sequence, treatment concerning the dose error and participant number.

13. Prior and concomitant medications

13.1 Variables and derivations

Prior and concomitant medications will be coded using WHODrug Global. See relevant Data Management Plan for details.

Medications taken by or administered to the participant for the period before screening will be recorded in the Prior & Concomitant Medications eCRF. Restricted therapies include over-the-counter medication within 7 days or prescription medication within 14 days prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, thyroid placement, or birth control methods).

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) other than NKF-INS(G), US-Lantus® and EU-Lantus® that the participant is receiving at the time of enrolment, or receives during the study, must be recorded in the eCRF as concomitant medications along with:

- Indication for use,
- Dates of administration including start and end dates,
- Dosage information including dose, regimen, route and frequency.

Imputations for partial start and/or end dates will be performed where at least the year is provided. The imputations derived for partial dates will be as follows:

- Missing days will default to the first of the month for start dates and the last day of the month for stop dates.
- Missing months will default to the first month (January) for start dates and to the last month (December) for stop dates.
- There will be no default for a missing year field.

Relevant prior medication given within 3 weeks before screening and all concomitant medication given from screening until the EOS visit (within 2-11 days after last administration of the study drug) must be recorded.

The participant must be told to notify the investigational site about any new medications taken after administration of the study treatment. The study treatment period will be derived as the last study treatment period the

participant was exposed to prior to starting their concomitant medication. All previous and on study Coronavirus disease 2019 (COVID-19) vaccinations should be recorded in the eCRF.

Other than those medications explicitly prohibited (*please refer to Section 8.6.1 in the CSP of NKF-INS(G)-101, v3.0 29-Aug-2024*), concomitant medication decisions will be left to the discretion of the Investigator.

13.2 Analysis

Prior and concomitant medications will be summarized by treatment sequence for the SAF. Prior and concomitant medications will also be presented in data listings by study treatment period per participant, for the RAS.

14. Adverse events

14.1 Variables and derivations

AEs will be coded using MedDRA. See the relevant Data Management Plan for details.

Treatment-emergent adverse events (TEAEs) are defined as AEs that started at the time of, or after the first study treatment administration as well as those events that started prior to the first study treatment administration, but which worsened after the first study treatment administration.

Imputations for start and stop dates will only be performed where at least the year is provided. The imputations derived for partial dates will be as follows:

- Missing days will default to the first of the month for start dates (or the day the participant received their first study treatment, if the first of the month is prior to this date and therefore does not meet the definition of an AE) and the last day of the month (or the day on which the participant had their last study visit, if this is before the last day of the month) for stop dates.
- Missing months will default to the month in which the participant received their first study treatment for start dates and to the month in which the participant had their last study visit for stop dates.
- There will be no default for a missing year field.

In the case where it is impossible to define an AE as treatment-emergent or not, the AE will be classified by the worst case assigned, i.e. a TEAE.

Similar imputations will be performed for the following:

- For an AE with missing severity, severity will be imputed as a Common Terminology Criteria for Adverse Events (CTCAE) severity of Grade 3 / Severe,
- For an AE with missing assessment of relationship to study treatment, relationship will be imputed as Related.

Adverse event summaries will be stratified by study treatment of event occurrence. *"Study treatment of event emergence"* will be derived as the

last study treatment the participant was exposed to prior to the onset of the AE.

If, due to missing dates, it is not possible to assign a TEAE to a study treatment of event emergence, the following imputations will be done:

- If day is missing and imputed as above, and month and year are recorded, the TEAE will be assigned to the study treatment received during that month. If more than one study treatment were received during that month, the TEAE will be assigned to all the study treatments received in that month.
- If both day and month are missing and imputed as above, the TEAE will be assigned to all the study treatments received during the study.

14.2 Analysis

Summaries will be presented as the number of participants within each of the event type categories described in the sub-sections below, including the incidence of TEAEs by SOC and preferred term, unless otherwise specified. Both number of participants, percentage of participants who experienced the event, as well as number of events will be provided at the level summarized.

For summaries presenting participant incidence, participants are counted only once per preferred term and study relationship to treatment and seriousness. For incidence per severity, participants are counted under each severity of an AE experienced (as recorded on the AE eCRF), as each AE is recorded with only one severity. All events are included in event counts in summaries and listings.

All AEs will also be listed by participant, along with information regarding onset, duration, severity, relationship to study treatment, action taken with study treatment, treatment of event, and outcome as captured in the eCRF.

14.2.1 Overview of TEAEs

An overview of all TEAEs will be presented as follows:

- Total number of AEs and TEAEs
- Total number of participants with at least one AE
- Total number of participants with at least one TEAE
- Total number of participants with CTCAE Grade ≥ 3 TEAEs
- TEAEs by relationship to study treatment
- Number of participants with TEAEs per CTCAE grade
    - Participants with multiple events per same CTCAE grade are counted only once per grade
- Number of participants with SAEs
- Number of participants with AEs leading to early withdrawal from study

For this Overview of TEAEs summary participants with multiple events in the specific bulleted category, is counted only once per category.

14.2.2 Incidence of TEAEs

The incidence of all TEAEs by SOC and preferred term will be presented by study treatment of event emergence.

14.2.3 TEAEs by severity

The Investigator will use the National Cancer Institute (NCI) CTCAE version 5.0 to describe the severity of AEs. AEs not listed in the CTCAE will be graded as follows:

- **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). ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
- **Grade 3**: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. Self-care ADL refers to bathing, dressing & undressing, feeding self, using the toilet, taking medications, and not bedridden.
- **Grade 4**: Life-threatening consequences; urgent intervention indicated.
- **Grade 5**: Death related to the AE.

The incidence of all TEAEs will be presented by SOC, preferred term and severity for each study treatment of event emergence.

14.2.4 Drug-related TEAEs

Classification of TEAEs by relationship to study treatment:

- NOT RELATED: There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.
- RELATED: The AE is known to emerge with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.

The incidence of all drug-related TEAEs will be presented by SOC and preferred term for each study treatment of event emergence.

14.2.5 Insulin Pen Adverse Events

The incidence of all TEAEs with “Other Etiology” indicated as “Insulin Pen” will be presented by preferred term and severity for each study treatment of event emergence.
All Insulin Pen AEs will be listed by participant, along with information regarding onset, duration, severity, date and study treatment of latest exposure, and outcome as captured in the eCRF.

14.2.6 Serious TEAEs

Any untoward medical occurrence, in the view of the Investigator, that:

- Results in death
- Is life-threatening: an AE is life-threatening if the participant was at immediate risk of death from the event as it occurred (i.e., it does not include a reaction that might have caused death if it had occurred in a more serious form).
- Requires or prolongs inpatient hospitalization: A hospitalization is defined as ≥ 24 hours in hospital or overnight stay. Complications occurring during hospitalization are AEs and SAEs if they cause prolongation of the current hospitalization. Hospitalization for elective treatment of preexisting, non-worsening conditions is not, however, considered an AE. The details of such hospitalizations must be recorded in the medical history/procedures or physical examination page of the eCRF.
- Results in persistent or significant disability/incapacity: an AE is incapacitating or disabling if it results in a substantial and/or permanent disruption of the participant's ability to carry out normal life functions.
- Results in a congenital anomaly/birth defect.
- Is another medically important event

  Other important medical events that may not be immediately life threatening or result in death or hospitalization, based upon appropriate medical judgement, are considered SAEs if they are thought to jeopardize the participant and/or require medical or surgical intervention to prevent one of the outcomes defining an SAE.

The incidence of all serious TEAEs will be presented by SOC and preferred term for each study treatment of event emergence. A data listing of SAEs will also be presented.

14.2.7 TEAEs leading to early withdrawal from study

A TEAE leading to early withdrawal from study is defined as a TEAE that occurs in a participant following which, in the Investigator's judgement, continued participation would pose unacceptable risk to the participant or to the integrity of the study data.

The incidence of all TEAEs leading to early withdrawal will be presented by SOC and preferred term for each study treatment of event emergence. A data listing of AEs leading to early withdrawal from the study will also be presented.

14.2.8 TEAEs leading to death

TEAEs leading to death are defined as TEAEs where the outcome was fatal during the study.

A data listing of all AEs for deceased participants will be presented.

15. Safety laboratory tests

All summaries for safety laboratory tests will be based on the SAF, unless otherwise specified. Data listings will also be based on the SAF.

Laboratory assessments may be repeated once during screening at the discretion of the investigator.

Any new or worsening laboratory test result considered by the Investigator to be clinically significant after screening will be considered an AE (clinically significant AEs include those that require intervention). Clinically significant abnormal values occurring during the study will be followed up until repeat test results return to normal, stabilize, or are no longer clinically significant.

15.1 Variables and derivations

For safety laboratory data, baseline will be defined as the last observation made prior to the first administration of study treatment.

The following laboratory tests (hematology, clinical chemistry, blood coagulation and urinalysis) to be included in the analysis:

15.1.1 Hematology

Ethylenediaminetetraacetic acid (EDTA) tubes:

- Full and differential blood count,
- White blood cell (WBC) count,
- Red blood cell (RBC) count,
- Hemoglobin (Hb),
- Hematocrit (HCT),
- Mean corpuscular volume (MCV),
- Mean corpuscular hemoglobin (MCH),
- Mean corpuscular hemoglobin concentration (MCHC),
- Absolute differential count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) and
- Platelets

15.1.2 Clinical Chemistry

Serum separator tubes (SSTs):

- Potassium,
- Sodium,
- Urea,
- Chloride,
- Phosphorus,
- Creatinine,
- Calcium,
- Albumin,
- Total and direct bilirubin,
- Creatine kinase (CK),
- Alkaline phosphatase (ALP),
- Gamma glutamyl transferase (GGT),
- Aspartate aminotransferase (AST),
- Alanine aminotransferase (ALT),
- Lactate dehydrogenase (LDH),
- Total cholesterol,
- Triglycerides, and
- Glucose.

15.1.3 Blood coagulation tests

Citrated tubes:

- Prothrombin time (PT),
- Activated partial thromboplastin time (aPTT), and
- International normalized ratio (INR)

15.1.4 Urinalysis

- Appearance,
- Glucose,
- Bilirubin,
- Ketone,
- Specific gravity,
- Blood,
- pH,
- Protein,
- Urobilinogen,
- Nitrite, and
- Leucocytes.

Abnormal urinalysis results may be repeated at the discretion of the investigator.

Quantitative laboratory measurements reported as "< X.XX", i.e., below limit of quantification (BLQ), or "> X.XX", i.e., above the upper limit of quantification, will be converted to X.XX for quantitative summaries, but will be presented as recorded, i.e., as "< X.XX" or "> X.XX" in the data listings.

15.2 Analysis

The SAF will be used to analyse safety laboratory data.

Summaries of hematology, clinical chemistry, blood coagulation and urinalysis laboratory tests for each treatment sequence, by visit and by time point, will include descriptive statistics of the following:

- Actual and CFB (for quantitative measurements)
- Frequencies and percentages (n and %) (for qualitative measurements)

Quantitative laboratory measurements will be compared with the relevant laboratory reference ranges and categorised as:

- Low: Below the lower limit of the laboratory reference range
- Normal: Within the laboratory reference range (upper and lower limit included)
- High: Above the upper limit of the laboratory reference range

These categorizations will only be presented in data listings. Other laboratory results (apart from hematology, clinical chemistry and urinalysis laboratory tests), e.g., urine microscopy, will be listed. A separate listing containing clinically significant laboratory results will be presented.

16. Vital signs

All summaries, and data listings, for vital signs will be based on the SAF, unless otherwise specified.

Vital signs will be measured after the participant has been resting quietly in a seated position for at least 5 minutes. Vital sign measurements will be repeated if clinically significant abnormal or machine/equipment errors occur. Out-of-range blood pressure, respiratory rate, or heart rate measurements may be repeated at the Investigator's discretion. Any confirmed, clinically significant abnormal vital sign measurements must be recorded as AEs.

16.1 Variables and derivations

For vital signs, baseline is defined as the last observation made prior to the first administration of IP.

The following vital signs will be reported for this study:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Heart rate (bpm)
- Body temperature (°C) (temporal or ear temperature measurements)
- Respiratory rate (breaths/min)

16.2 Analysis

The following summaries will be provided for vital signs data for each treatment sequence, by visit:

- Actual values and CFB by visit
- Descriptive statistics (n, arithmetic mean, SD, minimum, median, maximum) for absolute and CFB values.

Summaries of vital signs data will be presented by treatment sequence, for each study visit. Vital signs data will be listed per participant, as actual values, per visit and treatment sequence indicated.

17. Other assessments

The following assessments will be presented as limited summaries, summaries not including a CFB and/or only in data listings, for the SAF:

17.1 Physical examination data

A complete physical examination will be performed at screening (Visit 1). Physical examinations will be performed by a physician.

A full physical examination will include the following: evaluation for jaundice, pallor (anemia), cyanosis, clubbing, edema, and lymphadenopathy; skin evaluation: fundoscopy; ear, nose, and throat; cardiovascular assessment; respiratory assessment; abdominal examination; musculoskeletal assessment; neurological assessment. Other evaluations may be performed as deemed necessary by the Investigator. Full physical exam should be done at screening. A limited symptom-based physical exam may be done at EOS.

Physical examination data will be assessed as normal, abnormal clinically significant and abnormal not clinically significant.

Clinically significant findings must be recorded as AEs.

Physical examination data will be presented as a summary by treatment sequence, for screening and EOS visits, per body system and normal, abnormal clinically significant and abnormal not clinically significant assessment. Physical examination data will also be listed.

17.2 Electrocardiograms

A 12-lead, resting ECG will be obtained at the timepoints indicated in the schedule of assessments (*Table 3 in the CSP for NKF-INS(G)-101*). The ECG should always be performed after the participant had been resting in a supine position for at least 5 minutes.

An assessment of normal, abnormal clinically significant, or abnormal not clinically significant will be recorded; if the ECG is considered abnormal, the abnormality will be documented in the eCRF. ECGs may be repeated if clinically significant abnormalities are observed, or artifacts are present. Any confirmed, clinically significant abnormal ECG findings must be recorded as AEs.

Participant ECG status (normal, abnormal clinically significant, or abnormal not clinically significant) may be summarized for each scheduled ECG and shifts from screening may be tabulated. Raw data and derivations will also be presented as a listing for the SAF. Data will be analysed as outlined in ICH E14.

Abnormal ECG values will be identified in accordance with the following predefined normal ranges:

- PR Interval: 120 to 220 (msec)
- QRS Duration: < 120 (msec)
- Heart rate: 40 to 100 (bpm)
- QTc Interval: 350 to 450 (msec)
- For the QT, QTcF parameters, the following categorisations will be done:
  - x <= 450 ms (considered as normal range)
  - 450 < x <= 480 ms
  - 480 < x <= 500 ms
  - x > 500 ms
- For the PR parameter, the following categorisations will be done:
  - x < 120 ms
  - 120 <= x <= 220 ms (considered as normal range)
  - x > 220 ms
- For heart rate, the following categorisations will be done:
  - x < 50 bpm
  - 50 <= x <= 100 bpm (considered as normal range)
  - x > 100 bmp

A shift table will be presented for above categorizations, from screening to EOS, per treatment sequence.

17.3 Injection site assessment

The injection site and surrounding area will be assessed by the investigator at 1 min and 15 min post-dose on Day 1, and at the EOS visit.

The injection site assessment will be summarized (frequency and percentage) by type of assessment and timepoint (per study treatment for the 1 min and 15 min assessments), for the SAF. The injection site assessment will also be listed for the SAF.

17.4 Serology

Tests for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), immunoglobulin M antibody to Hepatitis B Core Antigen (IgM anti-HBc) and hepatitis C virus (HCV) will be performed at screening.

Results will be listed for the SAF.

17.5 Urine Drug Screen

A rapid, one-step screening test for simultaneous, qualitative detection of multiple drugs and drug metabolites will be used. Drugs-of-abuse testing will be performed at screening, and on admission (Day -1) to each treatment period. Participants with alleged false positive test results will be excluded from the study. A positive test may be repeated once at the discretion of the investigator.

Results will be listed for the SAF.

17.6 Alcohol Breath Test

An alcohol breath test using a portable breath alcohol measuring device will be performed at screening, on admission (Day -1) to each treatment period and at random. If any of these tests are positive, the participant will not be allowed further participation in the study.

Results will be listed for the SAF.

17.7 Human insulin antibodies

Participants will be tested for human insulin antibodies at screening.

Results will be listed for the SAF.

17.8 Oral glucose tolerance test

An oral glucose tolerance test will be conducted per site SOP. Oral glucose tolerance testing may be completed after all other eligibility has been determined.

Results will be listed for the SAF.

18. Pharmacokinetic Analysis

The primary objective of this study is to compare the PK of NKF-INS(G) to US-approved (US-Lantus®) and EU-authorized (EU-Lantus®) insulin to demonstrate PK similarity for the primary PK endpoint of $AUC_{0\text{-}24h}$ for M1. The secondary PK objective is to evaluate additional PK parameters NKF-INS(G) compared to US-approved and EU-authorized insulin glargine. Details on the endpoints are provided in Section 2.

PK concentrations and parameter data will be listed and summarized descriptively (N, arithmetic and geometric mean, SD, CV%, median, minimum and maximum) and graphically by treatment group as received.

Biosimilarity between the test product and the US-approved and EU-authorized insulin glargine will be considered separately, i.e., test product vs US-approved product and test product vs EU-authorized product. Biosimilarity of the test and reference products will be assessed on the basis of 90% CIs for estimates of the geometric mean ratios between the primary PK parameters of the test and reference products in relation to the conventional bioequivalence range of 80.00% to 125.00%.

Due to different regulatory requirements in terms of preferred statistical methods used in bioequivalence assessment, the test and respective reference insulin products will be analysed using two statistical models. Briefly, the Test - EU Reference comparison will be analysed using a model that only includes fixed effects and the Test – US-reference comparison will be analysed using a mixed effects model that includes both fixed and random effects.

18.1 Pharmacokinetic Concentrations

All PK concentrations, blood sampling times, including time deviations, will be listed for all participants in the SAF (to ensure data for participants excluded from the PK analysis set are listed).

For geometric statistics, imputed BLQ values of zero will be omitted. In addition, the percentage and number of participants, with plasma concentrations below the BLQ will be presented at each timepoint. Natural log transformations will be used throughout the analysis, where applicable. CV% will be calculated as follows:

Arithmetic CV%$= \left(\frac{arithmetic\ standard\ deviation}{arithmetic\ mean}\right) * 100$

Geometric CV% $= (\sqrt{\exp(variance) - 1}) * 100$

18.2 Pharmacokinetic Parameters

The PK parameters will be derived for NKF-INS(G), US-Lantus®, and EU-Lantus® from plasma concentrations by a validated PK analysis using linear up/ log down NCA (where data allow). A description of the PK parameters is provided in Table 18.2.

The below PK parameters will be calculated for the M1, M2 metabolites and glargine insulin. The PK parameters will be summarized for the analytes separately.

Table 18.2: PK Parameters and their descriptions.

PK Parameter	Description
AUC_{0-12h}	concentration-time curve from 0 to 12 hours
AUC_{0-6h}	concentration-time curve from 0 to 6 hours
AUC_{6-12h}	concentration-time curve from 6 to 12 hours
AUC_{12-18h}	concentration-time curve from 12 to 18 hours/end of clamp
AUC_{0-24h}	concentration-time curve from 0 to 24 hours/end of clamp
AUC_{12-24h}	concentration-time curve from 12 to 24 hours/end of clamp
AUC_{18-24h}	concentration-time curve from 18 to 24 hours/end of clamp
$AUC_{0-\infty}$	concentration-time curve from 0 hour to infinity
T_{max}	time to maximum serum concentration
C_{max}	maximum observed insulin Glargine concentration
$t_{1/2}$	terminal elimination half-life
λz	apparent terminal elimination rate constant
MRT	mean residence time
CL/F	total body clearance
V/F	volume of distribution

Reliability of parameter estimates:

- The percentage of the extrapolated AUC (area under the curve) should not exceed 20% of AUC for an individual profile. For profiles where the percentage of extrapolated AUC is greater than 20%, the observations should be flagged in the Analysis Data Model (ADaM) Pharmacokinetic Parameter (ADPP) dataset as unreliable, if applicable. Such observations should be listed, where possible, but not used in the preparation of summary statistics or any other statistical procedures.
- For terminal half-life, the following conditions should be met:
  - It should be determined over a time interval equal to at least 1.5 times the terminal half-life.
  - It should be estimated from at least 3 data points (primarily monotonically decreasing in magnitude), of which Clast (last observed quantifiable concentration) should be included, but not the datapoint at Tmax.
- The adjusted coefficient of determination (adj-R2) for the regression/fit should be at least 0.85.

If any one of the conditions are not met, an observation should be flagged as unreliable and listed, where possible, but not used in the preparation of summary statistics or any other statistical procedures. The observation should not be used in the Bioequivalence assessment, unless specified otherwise.

18.3 Bioequivalence assessment (EU)

As specified in the *Guideline on the investigation of bioequivalence* [7] the test product will be compared to the reference product by means of statistical analysis with respect to the primary PK parameter using an analysis of variance (ANOVA) with sequence, subject (sequence), treatment and period as fixed effects after logarithmic transformation of the relative PK parameter. Point estimate and two-sided 90% CI for the "test/EU-reference" geometric mean ratio of this parameter will be provided.

Bioequivalence will be established on the basis of the two-sided 90% CIs for estimates of the geometric ratios between the primary PK parameter of the test and EU-approved reference products being contained within the acceptance range of 80.00% to 125.00%. The primary PK parameter that will be used for the bioequivalence assessment is AUC_{0-24} of M1. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level.

18.4 Bioequivalence assessment (US)

As specified by *Guidance for industry: Statistical Approaches to Establishing Bioequivalence* [8] the test product will be compared to the reference product by means of statistical analysis with respect to the primary PK parameters using a linear mixed effects model following logarithmic transformation of the data. PK parameters will be estimated using treatment, period and sequence as fixed effects and participants as random effects. A difference in test and reference means and accompanying two-sided 90% CI will be estimated with the test/US-reference ratio of geometric means. Inter- and intra-subject variability will be estimated from the linear mixed effects model.

Point estimate and two-sided 90% CI for the "test/US-reference" geometric mean ratio of this parameter will be provided. The primary PK parameter that will be used for the bioequivalence assessment AUC_{0-24} of M1. All other PK-related assessments of equivalence will be considered secondary.

All secondary and exploratory PK parameters, for the test and US and EU reference analytes will also be presented. Bioequivalence for test/US-reference comparison in respect of Tmax will be assessed using a Wilcoxon signed rank test using the Hodges-Lehmann estimator. Bioequivalence assessment for test/US-reference comparison of the other secondary and exploratory PK parameters will be assessed as specified in Section 18.4. Bioequivalence assessment of the test/EU-reference comparison of the other secondary and exploratory PK parameters will be assessed as specified in Section 18.3.

Any missing samples or non-reportable concentration values will be disregarded in the PK analysis.

If terminal phase of serum concentration-time profile has sufficient data points, apparent terminal elimination half-life ($t^1/_2z$), apparent terminal elimination rate constant (λz), mean residence time (MRT), clearance (CL/F), and volume of distribution (V/F) will be calculated.

19. Pharmacodynamics Analysis

The PD objective is to compare the PD of NKF-INS(G) to US-approved (US-Lantus®) and EU-authorized (EU-Lantus®) insulin glargine injection by examining GIR profiles after a single SC dose. The key PD parameter for the analysis of the secondary objective is $GIRAUC_{0\text{-}24h}$. All other PD parameters will be considered as part of the secondary endpoints. Details on the endpoints are provided in Section 2.

PD GIR and parameter data will be listed and summarized descriptively (N, arithmetic and geometric mean, SD, CV%, median, minimum and maximum) for the PDPS by treatment group as received, and additionally graphically presented.

As for PK similarity, PD biosimilarity between the test product and the US-approved and EU-authorized insulin will be considered separately, i.e., test product vs US-approved product and test product vs EU-authorized product.

Due to different regulatory requirements in terms of preferred statistical methods used in bioequivalence assessment, the test and respective reference insulin products will be analysed using two statistical models. Briefly, the Test - EU Reference comparison will be analysed using a model that only includes fixed effects and the Test – US-reference comparison will be analysed using a mixed effects model that includes both fixed and random effects.

All PD analyses will be based on the PD parameter analysis set.

19.1 Pharmacodynamic Glucose Infusion Rates

Blood samples for glucose and GIR values will be collected following exposure to NKF-INS(G), US-Lantus®, and EU-Lantus® at the time points indicated in the sample schedule (*refer to Table 4; protocol NKF-INS(G)-101, Version 3.0, dated 29-Aug-24*). The actual date and time of each blood sample and GIR changes will be recorded. Actual time will be used for the derivation of PD parameters.

19.2 Pharmacodynamic parameters

The PD similarity will be assessed using the parameters provided in Table 19.2 and by assessing:

Table 19.2: PD Parameters and their descriptions.

PD Parameter	Description
$GIRAUC_{0\text{-}6h}$	Area under the GIR-time curve from 0 to 6 hours
GIR_{max}	The maximum GIR of glucose
$GIRAUC_{6\text{-}12h}$	Area under the GIR-time curve from 6 to 12 hours
$GIRAUC_{0\text{-}12h}$	Area under the GIR-time curve from 0 to 12 hours

$GIRAUC_{12\text{-}18h}$	Area under the GIR-time curve from 12 to 18
$GIRAUC_{18\text{-}24h}$	Area under the GIR-time curve from 18 to 24 hours
$GIRAUC_{12\text{-}24h}$	Area under the GIR-time curve from 12 to 24 hours
$GIRAUC_{0\text{-}24h}$	Area under the GIR-time curve from 0 to 24 hours
$GIRAUC_{0\text{-}\infty}$	Area under the GIR-time curve from 0 hours to the last quantifiable concentration time with extrapolation to infinity
$GIRAUC_{0\text{-end of clamp}}$	Area under the GIR-time curve from 0 hours to end of clamp
$TGIR_{max}$	Time to maximum GIR
G_{tot}	total amount of glucose infused during clamp procedure

Estimates of PD parameters will be listed and summarized (N, arithmetic and geometric mean, SD, CV%, median, minimum and maximum) by treatment group, descriptively and additionally presented graphically.

Endogenous insulin suppression via C-peptide measurements will also be explored. These values will include uncorrected C-peptide concentrations.

19.3 Bioequivalence assessment (EU)

As specified in the *Guideline on the investigation of bioequivalence* [7] the test product will be compared to the EU-reference product by means of statistical analysis with respect to the PD parameters using an ANOVA with sequence, subject (sequence), treatment and period as fixed effects after natural logarithmic transformation of the relative PD parameter. Point estimates and two-sided 95% CIs for the “test/EU-reference” geometric mean ratios of these parameters will be provided.

Biosimilarity will be assessed on the basis of the two-sided 95% CI for estimate of the geometric ratio between the key PD parameter of the test and EU-approved reference products being contained within the acceptance range of 80.00% to 125.00%. The PD parameter that will be assessed for bioequivalence as the key secondary endpoint is $GIRAUC_{0\text{-}24}$ and will be the first secondary endpoint assessed in the fixed sequence method. In addition, PD parameters listed in Table 19.2 will be assessed for bioequivalence.

19.4 Bioequivalence assessment (US)

As specified by *Guidance for industry: Statistical Approaches to Establishing Bioequivalence* [8] biosimilarity of the test and US-reference product will be assessed on the basis of two-sided 90% CI for estimate of the geometric mean ratio between the key PD parameter of the test and US-reference product in relation to the conventional bioequivalence range of 80.00% to 125.00%.

The test product will be compared to the reference product by means of statistical analysis with respect to the secondary PD parameters using a linear mixed effects model after logarithmic transformation of the data. PD parameters will be estimated using treatment, period and sequence as fixed effects and participants as random effects. A difference in test and US-reference means and accompanying two-sided 90% CIs will be estimated

with the test/US-reference ratio of geometric means. Inter- and intra-subject variability will be estimated from the linear mixed effects model.

Point estimates and 90% (US-REF) / 95% (EU-REF) CIs for the "test/reference" geometric mean ratios of these parameters will be provided. The PD parameter that will be used for the bioequivalence assessment is considered the first secondary objective $AUCGIR_{0-24}$ and will be the first secondary endpoint assessed in the fixed sequence method. The PD parameters that will be used for the bioequivalence assessment include those listed in Table 19.2.

20. Euglycemic Clamp Procedure and Performance

NKF-INS(G), US-Lantus®, and EU-Lantus® will be administered using a euglycemic glucose clamp at targeted blood glucose concentrations. The euglycemic glucose clamp will be conducted using a manual clamp technique, which will continuously monitor the participant's blood glucose to inform adjustment of the GIR to maintain blood glucose close to the target blood glucose concentration. The clamp procedure will be initiated, maintained, and terminated, in accordance with the site SOPs. The participant's blood glucose will be maintained within a predefined target window per site SOP and clinical judgement for the participant by manually adjusting the GIR.

The Target Glucose Value (TGV) for each participant is derived as follows (as described in the FARMOVS Standard Operating Procedure *Glucose Clamp Methodology CL14-v06 29-Jul-2024)*:

The TGV is derived as the mean baseline glucose value (mg/dL) subtracted by 5 mg/dL. The mean baseline glucose value will be derived as the mean (rounded to the whole number) of the glucose values obtained at the -60, -30, -15 and -5 time points.

The upper limit of the TGV is the mean baseline glucose value, and the lower limit value is the derived TGV subtracted by 5. As far as possible, the post dose glucose values of participants should be maintained within the upper and lower limit values by adjusting the GIR accordingly.

Blood samples will be collected at pre-specified intervals before and up to 24 hours after dosing for measurement of blood glucose, serum insulin, and C-peptide as shown in the schedule of assessments (*included in Table 4 of the SAP*).

High glucose clamp quality is important to precisely and reproducibly describe the time-action profiles of blood glucose (BG) lowering agents. To achieve high quality, that is, a GIR reflecting the correct PD effect, BG must be kept at the TGV as closely as possible. Glucose clamp quality will be examined to assess the validity of the Glucose Infusion Rate data obtained from the clamp procedures.

Clamp quality will be assessed as follows:

1. Graphically (blood glucose measurements plotted over time),
2. Variability of the blood glucose measurements are assessed (coefficient of variation) and,
3. The extent of the deviation of blood glucose measurements from the target value.

Calculations of the mean values, root mean square deviation, mean absolute relative difference (MARD) from the target value and CV of the BG concentrations will be used to provide an estimate of the performance of the clamp study. The mean ± SD difference between the clamp device and the target level reflects the "control deviation" and will be displayed in a figure. In this study, the blood glucose CV% will be considered as a guide of good clamp technique. A blood glucose CV% below 10% has been suggested [9] as the cut-off of sufficient clamp technique, i.e., CV % > 10% is considered poor clamp technique.

21. Multiplicity

To account for the multiple testing, a fixed sequence method will be used to control for multiplicity of the endpoint assessments. The fixed sequence below will be followed separately for the test/US-reference comparison and the test/EU-reference comparison. The endpoints are ordered as follows:

Primary Endpoint:

1. $AUC_{0\text{-}24h}$ of M1

Secondary Endpoints

(PK/PD parameters for plasma insulin glargine concentrations and/or M1):

2. Key secondary PD endpoint:
    a. $GIRAUC_{0\text{-}24h}$
3. $AUC_{0\text{-}12h}$
4. $GIRAUC_{0\text{-}12h}$
5. $AUC_{0\text{-}6h}$
6. $GIRAUC_{0\text{-}6h}$
7. $AUC_{6\text{-}12h}$
8. $GIRAUC_{6\text{-}12h}$
9. $AUC_{12\text{-}18h}$
10. $GIRAUC_{12\text{-}18h}$
11. $AUC_{12\text{-}24h}$
12. $GIRAUC_{12\text{-}24h}$
13. $AUC_{0\text{-}\infty}$
14. $GIRAUC_{0\text{-}\infty}$
15. $AUC_{18\text{-}24h}$
16. $GIRAUC_{18\text{-}24h}$
17. GIR_{max}
18. C_{max}
19. $TGIR_{max}$
20. T_{max}

21. $AUC_{0\text{-}24h}$ of insulin glargine

Testing will begin with the primary objective at the full alpha level and continue through the sequence until an assessment fails to reject the null hypothesis at which point further assessment of hypotheses in the sequence will be stopped.

The PK concentrations for M1, glargine and M2 will be tested in sequence separately. i.e., M1 will be considered first followed by glargine and then M2. The failure to reject a null hypothesis in the secondary M1 bioequivalence assessment endpoint sequence will not prohibit the analysis of glargine and M2.

Due to the risk of PK and PD parameters regarding the 18-24 hour interval being of poor reliability due to the less frequent sampling times as compared to earlier intervals or poorly populated due to early clamp terminations, the PK and PD parameters are to be examined later in the fixed sequence method than specified in the trial CSP.

22. References

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23. Revision history

Version	Date	Change
Version 1	13-May-2025	Initial Version
Version 2	29-July-2025	Minor spelling error and abbreviation fixes.