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

Protocol V3.0 29AUG2024 for NKF-INS(G)-101 is attached with NCT06627478.

CLINICAL STUDY PROTOCOL

Xentria, Inc.

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

Protocol Number: NKF-INS(G)-101

IND Number:	Pending
Name of Investigational Product:	NKF-INS(G) (Proposed Biosimilar to Insulin Glargine)
Phase of Development:	Phase 1
Indication:	Diabetes Mellitus
Sponsor:	Xentria, Inc. 2071 N. Southport Ave. Chicago, IL 60614 USA Tel: 224-443-4615 Email: contact@xentria.com
Protocol Version:	Version 3.0
Protocol Date:	29-Aug-2024

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PROTOCOL APPROVAL SIGNATURES

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
Protocol Number:	NKF-INS(G)-101

This study will be conducted in full conformance with the International Council for Harmonisation (ICH) E6 (R2) guideline for Good Clinical Practice (GCP) and the principles of the Declaration of Helsinki, or all relevant laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the European Union (EU) or European Economic Area will comply with the EU Clinical Trial Regulation (EU-CTR).

Sponsor Signatories:

Tom Matthews
Vice President, Clinical Development
Xentria, Inc.

Signature and Date

INVESTIGATOR SIGNATURE PAGE

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- I, the undersigned, have reviewed this protocol including appendices, and I will conduct the study as described in compliance with this protocol, GCP, and relevant ICH guidelines.
- I am thoroughly familiar with the appropriate use of the study treatment, as described in this protocol and any other information provided by Xentria, Inc. including, but not limited to, the current Investigator's Brochure.
- After the protocol has been approved by the Research Ethics Committee (REC)/Institutional Review Board (IRB), I will not modify this protocol. I will submit the protocol amendments and/or any informed consent form modifications to Xentria, Inc. and the REC/IRB, and approval will be obtained before any amendments are implemented.
- I ensure that all persons or parties assisting me with the study are adequately qualified, trained, and informed about the Xentria, Inc. study treatment and of their delegated study-related duties and functions as described in the protocol.
- I ensure that source documents and study records that include all pertinent observations on each of the site's study participants are attributable, legible, contemporaneous, original, accurate, complete, consistent, enduring, and available.
- I understand that all information obtained during the conduct of the study with regard to the participants' state of health will be regarded as confidential. No participants' personal identifying information will be disclosed. All participants will be identified by assigned numbers on all case report forms, laboratory samples, or source documents forwarded to the Sponsor. Clinical information may be reviewed by the Sponsor or its agents or regulatory agencies. Agreement must be obtained from the participant before disclosure of participant information to a third party.
- Information developed in this clinical study may be disclosed by Xentria, Inc. to other clinical Investigators, regulatory agencies, or other health authorities and government agencies as required.

Name:
Title:
Institution:

_____
Investigator Signature

_____
Date (DD-MMM-YYYY)

1. SYNOPSIS

Title of Study:	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
Protocol Number:	NKF-INS(G)-101
Investigational Therapy:	NKF-INS(G) administered as a 0.5 IU/kg single subcutaneous (SC) dose in the periumbilical area
Investigators/Study Site(s):	One center in South Africa.
Phase of Development:	Phase 1
Purpose and Rationale:	Despite advances in diabetes mellitus care, there remains a critical unmet need for cost-effective insulins in many markets. The number of individuals diagnosed with diabetes continues to rise, as does the prevalence of patients with uncontrolled diabetes, attributed partly to the costly nature of insulin. Diabetes places an economic burden on patients as well as the healthcare system (Heinemann, 2011). Currently available long-acting insulin analogues, while associated with a lower risk of hypoglycemia, are expensive. Insulin glargine is a long-acting insulin analogue marketed as Lantus® in the United States (US) and the European Union (EU) to improve glycemic control in adults and pediatrics above 2 years of age with type 1 diabetes mellitus and adults with type 2 diabetes mellitus. The release profile of insulin glargine resembles that of endogenous insulin secretion, providing a convenient method of insulin replacement with low risk of nocturnal hypoglycemia (Bhatia, 2021). As a proposed biosimilar to insulin glargine, NKF-INS(G) has the potential to address this unmet medical need in patients with diabetes mellitus. In order to demonstrate biosimilarity of NKF-INS(G) to reference products US-Lantus and EU-Lantus, a Phase 1 study will compare the pharmacokinetics (PK) and pharmacodynamics (PD) of NKF-INS(G) to the reference products using a euglycemic clamp technique in healthy male volunteers.

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_{0-24h} 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 concentrations and/or M1: C_{max}, AUC_{0-24h}, AUC_{0-6h}, AUC_{6-12h}, AUC_{0-12h}, AUC_{12-18h}, AUC_{18-24h}, and AUC_{12-24h} (fractional area under the plasma concentration-time curve), T_{max}, $AUC_{0-\infty}$
• To compare the PD of NKF-INS(G) to US-approved and EU-authorized Lantus by examining GIR profiles after a single SC dose.	• $GIRAUC_{0-24h}$, $GIRAUC_{0\text{-end of clamp}}$, $GIRAUC_{0-\infty}$, $GIRAUC_{0-6h}$, $GIRAUC_{6-12h}$, $GIRAUC_{0-12h}$, $GIRAUC_{12-18h}$, $GIRAUC_{18-24h}$, and $GIRAUC_{12-24h}$ (fractional area under the GIR-time curve), GIR_{max}, $TGIR_{max}$, and G_{tot}
• To assess the safety of NKF-INS(G)	• AE assessments (including injection site reactions), clinical laboratory investigations (hematology, clinical chemistry [including glucose], coagulation, and urinalysis), vital signs, physical examinations, 12-lead 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	• AUC_{0-24h}, C_{max}, and $AUC_{0-\infty}$ 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, $t_{½.z}$, λz, MRT, CL/F, and V/F will be calculated
• Measurement of assay sensitivity of plasma concentrations using LC-MS/MS data	• Comparative analysis of LC-MS/MS plasma concentration profiles

Abbreviations: AE: adverse event; 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_{0-24h}: AUC from 0 h to 24 h; $AUC_{0-\infty}$: AUC from 0 h to the last quantifiable concentration-time extrapolated to infinity; CL/F: total body clearance; C_{max}: maximum observed plasma exogenous insulin glargine concentration; ECG: electrocardiogram; GIR: glucose infusion rate; GIRAUC: area under the GIR-time curve (calculated as the exact area under the stepwise constant function); $GIRAUC_{0-24h}$: GIRAUC from 0 h to 24 h; $GIRAUC_{0-6h}$: GIRAUC from 0 h to 6 h; $GIRAUC_{6-12h}$: GIRAUC from 6 h to 12 h; $GIRAUC_{0-12h}$: GIRAUC from 0 h to 12 h; $GIRAUC_{12-18h}$: GIRAUC from 12 h to 18 h; $GIRAUC_{18-24h}$: GIRAUC from 18 h to 24 h; $GIRAUC_{12-24h}$: GIRAUC from 12 h to 24 h; $GIRAUC_{0-24h}$: GIRAUC from 0 h to 24 h;

$GIRAUC_{0-\infty}$: 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; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MRT: mean residence time; SC: subcutaneous; $t_{½.z}$: apparent terminal elimination half-life; PD: pharmacodynamic; PK: pharmacokinetic; T_{max}: time to maximum plasma exogenous insulin glargine concentration; T_{GIRmax}: time to maximum GIR; US: United States; V/F: volume of distribution; λ_z: apparent terminal elimination rate constant.	
Study Design:	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 US- and EU-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 to obtain informed consent and assess eligibility for participation, three dosing visits with a washout period between each visit, and a follow-up visit after the last dose. The entire study duration will be a maximum of 11 weeks. Three insulin products (NKF-INS(G), US-Lantus, and EU-Lantus) will be administered over three treatment periods. Upon admission to the clinical research unit (CRU) on Day -1, eligible participants will be randomized 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. A 24-hour euglycemic glucose clamp will be conducted using a manual clamp technique, which will monitor the subject's blood glucose and administer 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 dosing and until the end of clamp, up to 24 hours after dosing, for measurement of blood

	glucose, plasma insulin glargine (plus M1 and M2 metabolites), and serum C-peptide.
Selection of Participants:	**Inclusion Criteria** Individuals must meet all of the following inclusion criteria to be included in the study: 1. Signed and dated informed consent obtained before any trial-related activities. Trial-related activities are any procedures that would not have been done during normal management of the participant. 2. Healthy male participants 3. Age between 18 and 50 years, both inclusive 4. Body Mass Index between 18.5 and 29.0 kg/m^2, both inclusive 5. Body weight ≥ 59 kg 6. Fasting glucose concentration ≤ 5.5 mmol/L at screening 7. Considered generally healthy upon completion of medical history, physical examination, vital signs, electrocardiogram (ECG), and analysis of laboratory safety variables, as judged by the Investigator 8. Willing and able to comply with scheduled visits, treatment plan, clinical laboratory tests, and other study procedures including lifestyle considerations. 9. Participants must agree to use condoms during sexual intercourse. Additionally, female partners of male participants should use highly effective contraception. All contraceptive measures apply from screening until 90 days after study treatment. Male participants must refrain from donating or banking sperm for 90 days after administration of study treatment. 10. Have competence in speaking, writing, and comprehending the local language(s) where the study is conducted. **Exclusion Criteria** Individuals will be excluded from participation if they meet any of the following criteria: 1. Positive for human insulin antibodies at Screening 2. Are currently enrolled in or have discontinued within 3 months or 5 half-lives (whichever is longer) of any investigational drug or device or are concurrently enrolled in any other type of medical research study and judged not to be scientifically or medically compatible with this study.

	3. Have known allergies to insulin, its excipients, or related drugs or have history of relevant allergic reactions of any origin, or any specific investigational product safety concern. 4. History of diabetes mellitus; episodes of hypoglycemia in the anamnesis; any history of insulin use for treatment purposes. 5. Have clinically relevant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; of constituting a risk when taking the study drug; or of interfering with the interpretation of data. 6. Increased risk of thrombosis, e.g., individuals with a history of deep leg vein thrombosis or family history of deep leg vein thrombosis, as judged by the Investigator. 7. Clinically significant abnormal ECG at screening. 8. Glycemia level ≥140 mg/dL 2 hours after the glucose load. 9. Evidence of psychiatric disorder, antagonistic personality, poor motivation, emotional or intellectual problems likely to limit the validity of consent to participate in the study or limit the ability to comply with protocol requirements. 10. Positive urine drug test at screening and/or evidence of current use of known drugs of abuse or have a history of use within the past year. 11. Show evidence of an acute infection with fever or infectious disease at the time of enrollment. 12. Show evidence of human immunodeficiency virus (HIV) infection and/or positive human HIV antibodies at screening. 13. Have positive test results for hepatitis B surface antigen (HBsAg), immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc), or hepatitis C virus (HCV) antibodies at screening. 14. Intend to use over-the-counter medication within 7 days or prescription medication within 14 days prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, thyroid replacement). 15. Have donated blood or had a blood loss of 500 mL 3 months prior to study enrollment.

	16. Have an average weekly alcohol intake that exceeds 21 units per week or is unwilling to stop alcohol consumption from 48 hours prior to each dosing until being discharged from the CRU. 17. Employees or close relatives of the Sponsor, contract research organization (CRO), third-party vendors. or affiliates of the above-mentioned parties. 18. Inadequate venous access. 19. Vulnerable populations, e.g., persons in detention.
Planned Sample Size:	Based on a CV% of 43, 114 participants are planned to be enrolled to have at least 96 evaluable participants.
Investigational Medical Products **Study Product:** **Reference Therapy:**	 NKF-INS (G) administered as a 0.5 IU/kg single SC dose in the periumbilical area US-Lantus and EU-Lantus, both administered as a 0.5 IU/kg single SC dose in the periumbilical area
Treatment Duration:	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 within 2-11 days after the last dose administration of treatment period 3 or Early Termination. The entire study duration will be a maximum of 11 weeks.
Safety:	Safety assessments include vital signs, physical examinations, ECG recordings, clinical laboratory assessments (hematology, clinical chemistry [including glucose], coagulation, and urinalysis) and adverse event (AE) monitoring (including injection site reactions). All treatment-emergent adverse events (TEAEs) will be summarized by treatment group (NKF-INS(G), US-Lantus, and EU-Lantus) as follows: • All AEs • Grade ≥3 AEs • AEs related to study treatment, including related Grade ≥3 AEs • All adverse reactions • Serious adverse events (SAEs)

	All AEs will be listed by participant, along with information regarding onset, duration, relationship and severity to study treatment, action taken with study treatment, treatment of event, and outcome. Clinical laboratory data (hematology, clinical chemistry [including glucose], coagulation, and urinalysis) and vital signs (heart rate, respiratory rate, systolic and diastolic blood pressure, and temporal or ear temperature measurements) will be summarized by treatment group and scheduled visits using descriptive statistics, including mean values and mean change from screening values, as well as numbers of participants with values outside limits of the normal range at each timepoint. Shift tables will be provided presenting change from screening values to the End of Study (EOS) visit. The frequency of ECG abnormalities (e.g., heart rate, PR interval, QRS complex, and QT-interval corrected by Fridericia's formula [QTcF]) will be summarized by treatment group and scheduled visits using counts and percentages. 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. Injection site reactions will be summarized by treatment group and scheduled visits. The number and percentage of participants having reactions at each assessment timepoint, overall and by specific reaction subtype, will be presented. Summary tables will be provided for concomitant medications initiated during the study period. Clinically significant changes in physical examination findings and laboratory abnormalities will be listed as AEs.
Pharmacokinetics:	Insulin glargine (plasma) samples will be collected at 60 and 0 minutes pre-dose and 0.5, 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 24 hours post-dose.
Pharmacodynamics:	C-peptide (serum) samples will be collected at 60 and 0 minutes pre-dose and 0.5, 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 24 hours post-dose. Blood glucose values will be analyzed pre-dose at 60,

	30, 15, and 5 minutes (baseline is defined as the mean glucose at these 4 time points), at 0 min, every 5 minutes from 0 to 4 hours, and every 10 minutes from 4 hours until the end of clamp.
Statistical Methods and Planned Analyses:	The statistical evaluation will be performed using R version 4.0 or higher. All data will be listed, and summary tables and figures will be provided. Summary statistics will be presented by treatment group, to the extent possible. For continuous variables, data will be summarized with the number of participants (N), arithmetic mean, standard deviation (SD), median, minimum, and maximum by treatment group. Geometric mean and coefficient of variation (CV%) will be presented as appropriate, by treatment group. For categorical variables, data will be tabulated with the number and proportion of participants for each category by treatment group.

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3. LIST OF ABBREVIATIONS

Abbreviation	Expanded Term
ADL	Activities of Daily Living
AE	Adverse Event
ALT	Alanine Aminotransferase
Anti-HBc	Antibody to Hepatitis B Core Antigen
AST	Aspartate Aminotransferase
CFR	Code of Federal Regulations
CI	Confidence Interval
CL/F	Total Body Clearance
CMP	Clinical Management Plan
COVID-19	Coronavirus Disease 2019
CRO	Contract Research Organization
CRU	Clinical Research Unit
CTCAE	Common Terminology Criteria for Adverse Events
CTR	Clinical Trial Regulation
CV	Coefficient of Variation
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EOS	End of Study
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GIR	Glucose Infusion Rate
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ID	Identification
IgM	Immunoglobulin M
INR	International Normalized Ratio
IRB	Institutional Review Board
IU	International Units
MRT	Mean Residence Time
NCI	National Cancer Institute
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
QC	Quality Control
QTcF	QT-Interval Corrected by Fridericia's Formula

REC	Research Ethics Committee
SAE	Serious Adverse Event
SAHPRA	South African Health Products Regulatory Authority
SAP	Statistical Analysis Plan
SC	Subcutaneous
SD	Standard Deviation
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
t½.z	Apparent Terminal Elimination Half-Life
TEAE	Treatment-Emergent Adverse Event
ULN	Upper Limit of Normal
US	United States
USPI	United States Prescribing Information
V/F	Volume of Distribution
λz	Apparent Terminal Elimination Rate Constant

Note: Definitions of pharmacokinetic (PK) and pharmacodynamic (PD) parameters are provided in Section 10.1.2 and Section 11.1.2.

4. INTRODUCTION

Insulin therapy is essential for the treatment of diabetes mellitus type 1 and is often used in diabetes mellitus type 2. Insulin glargine is a basal insulin. The onset of action is approximately 3-4 hours, with a subtle peak at approximately 10-12 hours, and a duration of 24 hours or longer.

Currently available long-acting insulin analogues, while associated with a lower risk of hypoglycemia, are expensive (Ampudia Blasco, 2020). Insulin glargine is a long-acting insulin analogue marketed as Lantus® in the United States (US) and the European Union (EU). It has a subtle peak and a longer duration of action, resulting in daily glycemic control by means of lowering the total glucose throughout the day.

Biosimilar insulins should offer the advantages of insulin analogues at reduced costs.

4.1. Background on NKF-INS(G)

As a proposed biosimilar to insulin glargine, NKF-INS(G) has the potential to address the critical unmet need for cost-effective insulins in patients with diabetes.

To demonstrate biosimilarity compared to US-approved and EU-authorized Lantus, a comparative clinical pharmacology study between the proposed insulin glargine test product (NKF-INS(G)) and both US- and EU-Lantus reference products is required (FDA, 2015; FDA, 2016). This study should provide a time-concentration profile and a time-action profile over the duration of action of each product based on reliable measures of systemic exposure and glucose response.

As a proposed biosimilar to insulin glargine, NKF-INS(G) has the same amino acid sequence as US- and EU- Lantus. Insulin glargine differs from human insulin in that the amino acid asparagine at position A21 is replaced by glycine and two arginines are added to the C-terminus of the B-chain. NKF-INS(G) and Lantus are produced by recombinant DNA technology utilizing *Escherichia coli*. NKF-INS(G) has the empirical formula of $C_{267}H_{404}N_{72}O_{78}S_6$ and has a molecular weight of 6063 Da.

As in human insulin, insulin glargine contains two interchain disulfide bonds and one intrachain disulfide bond.

The primary activity of insulin glargine is the regulation of glucose metabolism. Insulins, including insulin glargine, bind to the insulin receptors on muscle and fat cells and lower blood glucose by facilitating the cellular uptake of glucose and simultaneously inhibiting the output of glucose from the liver.

This is the first clinical study of NKF-INS(G) drug product.

4.1.1. Preliminary Analytical Similarity Study for Program Feasibility

To confirm the feasibility with the Food and Drug Administration (FDA) for the licensure in the US of NKF-INS(G) as a biosimilar to US-licensed US-Lantus through the 351(k) pathway, a preliminary analytical similarity study was conducted between the reference product and NKF-INS(G) early development drug substance lots, utilizing a subset of quality attributes to be included in the full analytical similarity test panel. Three lots of US-licensed Lantus were included and three NKF-INS(G) early development drug substance lots were included in this similarity study. The most critical quality attributes that were not likely to be significantly

impacted by the drug product fill/finish process and container closure were included in this study.

The preliminary analytical similarity study results demonstrated that NKF-INS(G) is highly similar to the US-reference product in both physicochemical and biological characteristics, therefore further development of NKF-INS(G) as a biosimilar to US-Lantus is feasible.

No other nonclinical animal studies or clinical studies have been completed for NKF-INS(G).

4.2. Clinical Benefits/Risks of NKF-INS(G)

The safety profile of NKF-INS(G) has not been evaluated in humans. Based on currently approved insulin glargine products, potential side effects may include allergic reactions, hypoglycemia, injection site reactions, lipodystrophy, pruritus, rash, edema, hypokalemia, and weight gain. Recognizing that NKF-INS(G) is a biosimilar product, no additional risks are expected; however, participants will be closely monitored throughout the study for side effects.

Details regarding known or anticipated benefits and risks, as well as reasonably anticipated adverse events (AEs) for NKF-INS(G) are provided in the Investigator's Brochure.

4.3. Study Rationale

Despite advances in diabetes mellitus care, there remains a critical unmet need for cost-effective insulins in many markets. The number of individuals diagnosed with diabetes continues to rise, as does the prevalence of patients with uncontrolled diabetes, attributed partly to the costly nature of insulin. Diabetes places an economic burden on patients as well as the healthcare system (Heinemann, 2011).

Currently available long-acting insulin analogues, while associated with a lower risk of hypoglycemia, are expensive. Insulin glargine is a long-acting insulin analogue marketed as Lantus in the US and the EU to improve glycemic control in adults and pediatrics above 2 years of age with type 1 diabetes mellitus and adults with type 2 diabetes mellitus. The release profile of insulin glargine resembles that of endogenous insulin secretion, providing a convenient method of insulin replacement with low risk of nocturnal hypoglycemia (Bhatia, 2021).

As a biosimilar to insulin glargine, NKF-INS(G) has the potential to address this unmet medical need in patients with diabetes mellitus. In order to demonstrate biosimilarity of NKF-INS(G) to reference products US-Lantus and EU-Lantus, a Phase 1 study will compare the pharmacokinetics (PK) and pharmacodynamics (PD) of NKF-INS(G) to the reference products using a euglycemic clamp technique in healthy male volunteers (FDA, 2015; FDA, 2016).

4.4. Dose Rationale

NKF-INS(G), US-Lantus and EU-Lantus will be administered as a 0.5 IU/kg single subcutaneous (SC) dose in the periumbilical area. US-Lantus and EU-Lantus are dosed between 0.5 IU/kg/day and 1.0 IU/kg/day to patients with diabetes mellitus (Lantus United States Prescribing Information (USPI), 2023). Given this study will recruit healthy male participants, a dose of 0.5 IU/kg was selected and aligned with similar trials of glargine analogues (Bhatia, 2021; Chen, 2023; Linnebjerg, 2015).

NKF-INS(G) dosage is individualized based on the route of administration, and the participant's metabolic needs, blood glucose monitoring results, and glycemic control goal. For the purposes of the clinical study, the participant will receive a single dose of 0.5 IU/kg SC by injection in the periumbilical area in three treatment periods.

5. STUDY OBJECTIVES AND ENDPOINTS

Objectives and related endpoints are described in Table 1.

Table 1: 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_{0-24h} 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 concentrations and/or M1: C_{max}, AUC_{0-24h}, AUC_{0-6h}, AUC_{6-12h}, AUC_{0-12h}, AUC_{12-18h}, AUC_{18-24h}, and AUC_{12-24h} (fractional area under the plasma concentration-time curve), T_{max}, $AUC_{0-\infty}$
• To compare the PD of NKF-INS(G) to US-approved and EU-authorized Lantus by examining GIR profiles after a single SC dose.	• $GIRAUC_{0-24h}$, $GIRAUC_{0-end\ of\ clamp}$, $GIRAUC_{0-\infty}$, $GIRAUC_{0-6h}$, $GIRAUC_{6-12h}$, $GIRAUC_{0-12h}$, $GIRAUC_{12-18h}$, $GIRAUC_{18-24h}$, and $GIRAUC_{12-24h}$ (fractional area under the GIR-time curve), GIR_{max}, $TGIR_{max}$, and G_{tot}
• To assess the safety of NKF-INS(G)	• AE assessments (including injection site reactions), clinical laboratory investigations (hematology, clinical chemistry [including glucose], coagulation, and urinalysis), vital signs, physical examinations, 12-lead 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	• AUC_{0-24h}, C_{max}, and $AUC_{0-\infty}$ of M2

Objectives	Endpoints
• 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, $t_{½.z}$, λz, MRT, CL/F, and V/F will be calculated
• Measurement of assay sensitivity of plasma concentrations using LC-MS/MS data	• Comparative analysis of LC-MS/MS plasma concentration profiles

Abbreviations: AE: adverse event; 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_{0-24h}: AUC from 0 h to 24 h; $AUC_{0-\infty}$: AUC from 0 h to the last quantifiable concentration-time extrapolated to infinity; CL/F: total body clearance; C_{max}: maximum observed plasma exogenous insulin glargine concentration; ECG: electrocardiogram; GIR: glucose infusion rate; GIRAUC: area under the GIR-time curve (calculated as the exact area under the stepwise constant function); $GIRAUC_{0-24h}$: GIRAUC from 0 h to 24 h; $GIRAUC_{0-6h}$: GIRAUC from 0 h to 6 h; $GIRAUC_{6-12h}$: GIRAUC from 6 h to 12 h; $GIRAUC_{0-12h}$: GIRAUC from 0 h to 12 h; $GIRAUC_{12-18h}$: GIRAUC from 12 h to 18 h; $GIRAUC_{18-24h}$: GIRAUC from 18 h to 24 h; $GIRAUC_{12-24h}$: GIRAUC from 12 h to 24 h; $GIRAUC_{0-24h}$: GIRAUC from 0 h to 24 h; $GIRAUC_{0-\infty}$: 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; LC-MS/MS: liquid chromatography with tandem mass spectrometry; MRT: mean residence time; SC: subcutaneous; $t_{½.z}$: apparent terminal elimination half-life; PD: pharmacodynamic; PK: pharmacokinetic; T_{max}: time to maximum plasma exogenous insulin glargine concentration; T_{GIRmax}: time to maximum GIR; US: United States; V/F: volume of distribution; λ_z: apparent terminal elimination rate constant.

6. INVESTIGATIONAL PLAN

6.1. Description of Overall Study Design and Plan

The NKF-INS(G) clinical program is designed to demonstrate biosimilarity to US- and EU-insulin glargine (US-Lantus and EU-Lantus) by comparing the PK and PD of NKF-INS(G) to the reference products after a single SC dose.

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 US- and EU-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), US-Lantus, and EU-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.

A 24-hour euglycemic glucose clamp will be conducted using a manual clamp technique, which will monitor the subject's blood glucose and administer 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 dosing and until the end of clamp, up to 24 hours after dosing, for measurement of blood glucose, plasma insulin glargine, and serum C-peptide.

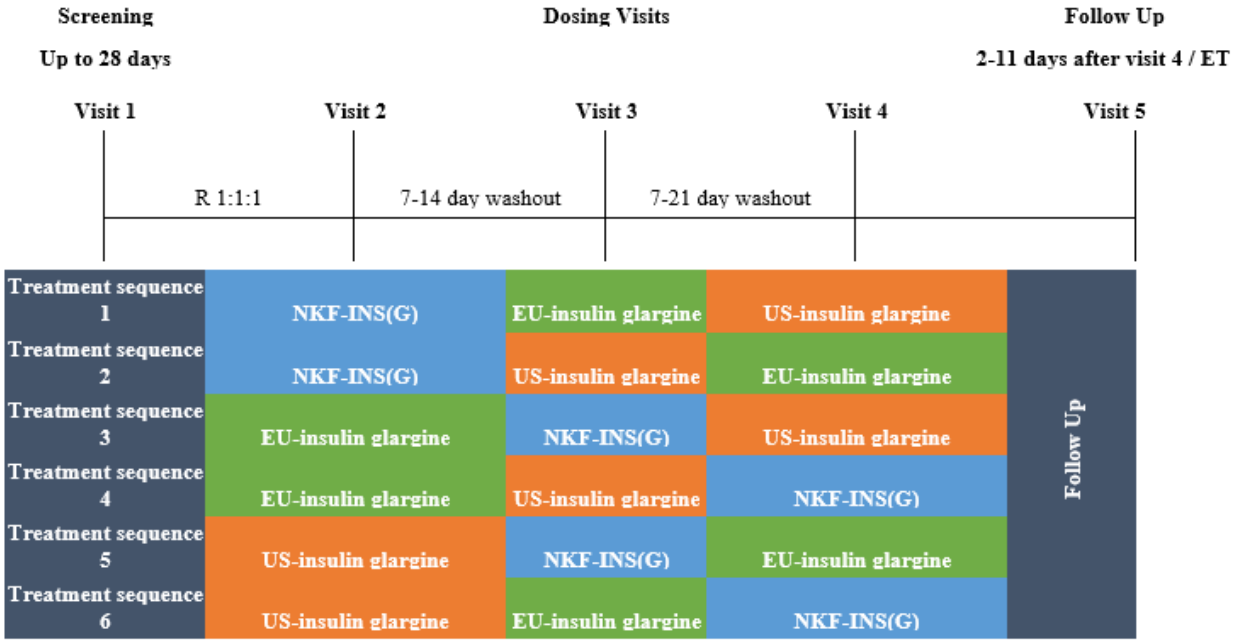
One hundred fourteen healthy male participants are planned to be enrolled in the study to have at least 96 evaluable participants.

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 period
- 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

The study design schema is provided in Figure 1.

Figure 1: Study Design



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

6.2. Discussion of Study Design

Congruent to Draft Guidance "*Clinical Immunogenicity Considerations for Biosimilar and Interchangeable Insulin Products"*, the Sponsor believes the aforementioned comparative analytical assessment demonstrates NKF-INS(G) is highly similar to US- and EU-Lantus and there is no residual uncertainty regarding immunogenicity. Therefore, in addition to the comparative analytical assessments, the Sponsor plans to conduct one Phase 1 study to demonstrate PK similarity to reference products to provide a totality of evidence for biosimilarity for licensure.

The design of the study was chosen as both FDA and European Medicines Agency recognize the clamp technique as the most reliable method for qualifying the PK and PD proprieties of a novel biosimilar. Clamp technique is used to rapidly attain the required blood glucose levels and maintain it approximately at target levels (Heinemann, 2023).

6.3. End of Study

A participant will have fulfilled the requirements for study completion if/when the participant has completed all three treatment periods and the end of study (EOS) visit within 2-11 days after last administration of the study drug.

The EOS is defined as the date of the last visit for the last participant in the study/last scheduled procedure shown in the schedule of assessments (Table 3).

6.4. Criteria for Early Study Termination

The Sponsor reserves the right to close a study site or terminate the study at any time for any reason. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, requirements of the Institutional Review Board (IRB)/Research Ethics Committee (REC) or local health authorities, the Sponsor's procedures, or ICH GCP guidelines, or contractual agreement.
- Inadequate recruitment of participants by the Investigator.
- Discontinuation of further development of NKF-INS(G).

The IRB/REC and regulatory authorities will be informed about the reason and time of the termination according to applicable laws and regulations. If the study is terminated prematurely, Investigators will inform their participants.

7. SELECTION OF STUDY POPULATION

7.1. Inclusion Criteria

Individuals must meet all of the following inclusion criteria to be included in the study:

1. Signed and dated informed consent obtained before any trial-related activities. Trial-related activities are any procedures that would not have been done during normal management of the participant.
2. Healthy male participants
3. Age between 18 and 50 years, both inclusive
4. Body Mass Index between 18.5 and 29.0 kg/m2, both inclusive
5. Body weight ≥ 59 kg
6. Fasting glucose concentration ≤ 5.5 mmol/L at screening
7. Considered generally healthy upon completion of medical history, physical examination, vital signs, electrocardiogram (ECG), and analysis of laboratory safety variables, as judged by the Investigator.
8. Willing and able to comply with scheduled visits, treatment plan, clinical laboratory tests, and other study procedures including lifestyle considerations.
9. Participants must agree to use condoms during sexual intercourse. Additionally, female partners of male participants should use highly effective contraception. All contraceptive measures apply from screening until 90 days after study treatment. Male participants must refrain from donating or banking sperm for 90 days after administration of study treatment.
10. Have competence in speaking, writing, and comprehending the local language(s) where the study is conducted.

7.2. Exclusion Criteria

Individuals will be excluded from participation if they meet any of the following criteria:

1. Positive for human insulin antibodies at Screening
2. Are currently enrolled in or have discontinued within 3 months or 5 half-lives (whichever is longer) of any investigational drug or device or are concurrently enrolled in any other type of medical research study and judged not to be scientifically or medically compatible with this study.
3. Have known allergies to insulin, its excipients, or related drugs or have history of relevant allergic reactions of any origin or any specific investigational product safety concern

4. History of diabetes mellitus; episodes of hypoglycemia in the anamnesis; any history of insulin use for treatment purposes.
5. Have clinically relevant history of or current cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrine, hematological, or neurological disorders capable of significantly altering the absorption, metabolism, or elimination of drugs; of constituting a risk when taking the study drug; or of interfering with the interpretation of data.
6. Increased risk of thrombosis, e.g., individuals with a history of deep leg vein thrombosis or family history of deep leg vein thrombosis, as judged by the Investigator.
7. Clinically significant abnormal ECG at screening.
8. Glycemia level ≥140.4 mg/dL 2 hours after the glucose load.
9. Evidence of psychiatric disorder, antagonistic personality, poor motivation, emotional or intellectual problems likely to limit the validity of consent to participate in the study or limit the ability to comply with protocol requirements.
10. Positive urine drug test at screening and/or evidence of current use of known drugs of abuse or have a history of use within the past year.
11. Show evidence of an acute infection with fever or infectious disease at the time of enrollment.
12. Show evidence of human immunodeficiency virus (HIV) infection and/or positive human HIV antibodies at screening.
13. Have positive test results for hepatitis B surface antigen (HBsAg), immunoglobulin M (IgM) antibody to hepatitis B core antigen (anti-HBc), or hepatitis C virus (HCV) antibodies at screening.
14. Intend to use over-the-counter medication within 7 days or prescription medication within 14 days prior to dosing (apart from vitamin/mineral supplements, occasional paracetamol, thyroid replacement).
15. Have donated blood or had a blood loss of 500 mL 3 months prior to study enrollment.
16. Have an average weekly alcohol intake that exceeds 21 units per week or is unwilling to stop alcohol consumption from 48 hours prior to each dosing until being discharged from the CRU.
17. Employees or close relatives of the Sponsor, contract research organization (CRO), third-party vendors, or affiliates of the above-mentioned parties.
18. Inadequate venous access.
19. Vulnerable populations, e.g., persons in detention.

7.3. Rescreening

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered into the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened only once, at the discretion of the Investigator.

The participant must be reconsented and a new screening number will be assigned. Also, a new informed consent form (ICF) must be signed if updates have been made since signing of the first ICF.

7.4. Study Withdrawal, Removal, and Replacement of Participants

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 (see Section 8.4).

If a participant withdraws from the study for any reason, the study site must immediately notify the clinical monitor. The date and the reason for study discontinuation must be recorded in the electronic case report form (eCRF). Participants who discontinue early, irrespective of the reason for discontinuation from the study, will be asked to complete the EOS assessments at the time of the participant's withdrawal, as outlined in the schedule of assessments (Table 3).

If a participant withdraws from the study, he may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records.

Additionally (as described in Section 6.4), the Sponsor may stop the study at any time for safety, regulatory, legal, or other reasons aligned with GCP. This study may be terminated at the discretion of the Sponsor or any regulatory agency. An Investigator may elect to discontinue or stop the study at his or her study site for any reason, including safety or low enrollment.

7.4.1. Replacement of Participants

Participants who sign the ICF and are randomized but not treated may be replaced at the discretion of the Sponsor.

8. TREATMENTS

A list of study treatments used in this study is provided in Table 2.

Table 2: Study Treatments

Agent	Use
NKF-INS(G)	Investigational agent
US-Lantus and EU-Lantus	Indicated to improve glycemic control in adults and children with diabetes mellitus

Source: Lantus USPI, 2023; Lantus Summary of Product Characteristics (SmPC), 2015

8.1. Study Treatment Description

NKF-INS(G), US-Lantus and EU-Lantus are intended to be administered as a 0.5 IU/kg single SC dose in the periumbilical area.

US-Lantus and EU-Lantus (sanofi-avenits, Bridgewater, NJ) will be supplied by sanofi-aventis, Bridgewater, NJ, US.

NKF-INS(G) is a clear and colorless solution.

NKF-INS(G) is manufactured and supplied in a disposable pen (with cartridge) containing 300 IU/3mL; 1 mL solution contains 100 units of NKF-INS(G), equivalent to 3.6 mg. The disposable pen syringe device is a BD Vystra Disposable Pen sourced commercially (Figure 2) and used with a NovoFine needle or equivalent (Figure 4).

Figure 2: Investigational Product Description



US-Lantus and EU-Lantus are supplied in a disposable pen syringe containing 300 IU/3mL (Lantus USPI, 2023; Lantus SmPC, 2015); 1 mL solution contains 100 units of insulin glargine equivalent to 3.6 mg. The disposable SoloStar pen is a proprietary device (Figure 3) with a NovoFine needle or equivalent (Figure 4).

Figure 3: Reference Product

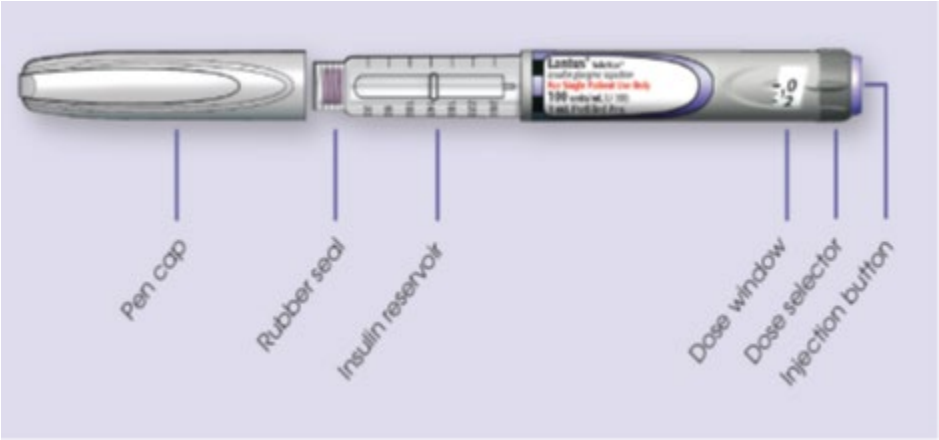
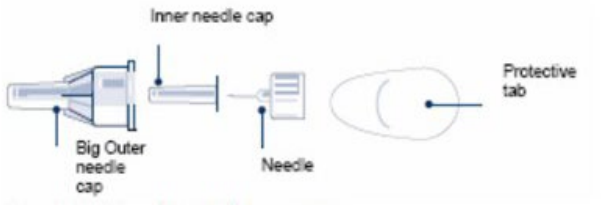


Figure 4: NovoFine Needle



8.1.1. Preparation, Handling, and Disposal

The preparation of NKF-INS(G), US-Lantus and EU-Lantus will be conducted in accordance with the Pharmacy Manual, USPI, and SmPC.

Procedures for proper handling and disposal should be followed in compliance with the SOPs of the site.

8.1.2. Product Labeling and Packaging

NKF-INS(G), US-Lantus and EU-Lantus will be labeled to meet country-specific regulatory requirements.

Refer to the Pharmacy Manual for packaging and labeling information.

8.1.3. Administration

NKF-INS(G), US-Lantus and EU-Lantus will be administered as a single dose via the SC route (refer to Section 8.2). The dose will be calculated by an unblinded pharmacist and confirmed by another unblinded pharmacist as described in the Pharmacy Manual. Device-related events or complaints should be reported in accordance with the Pharmacy Manual.

8.1.4. Storage

Refer to the Pharmacy Manual for information regarding storage, handling, and administration of NKF-INS(G), US-Lantus and EU-Lantus.

Before opening (unpunctured), NKF-INS(G) and reference products should be stored at 2 °C to 8 °C (36 °F to 46 °F) and protected from heat and light until the time of use. The drug product must not be frozen or placed adjacent to the refrigerator cooling element.

During use (punctured), NKF-INS(G) and reference products should be stored below 30 °C (86 °F) for up to 28 days and should not be exposed to excessive heat or sunlight. Do not freeze and do not draw into a syringe for later use. Pen cap should be kept on the pen in order to protect from light.

Always remove and discard the needle after each injection. Pens are considered single use.

NKF-INS(G) is being studied on an ongoing stability program and is tested at regular intervals to establish shelf life.

8.2. Dosage Schedule

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.

The insulin products will be administered as a single dose via the SC route. An euglycemic glucose clamp will be utilized thereafter at a targeted blood glucose concentration. The euglycemic glucose clamp will be conducted using a clamp technique. GIR will be manually adjusted to maintain the target blood glucose concentration based on the actual measured blood glucose concentration and the degree of variability in the previous minute.

Blood samples will be collected at pre-specified intervals before dosing and until the end of clamp, up to 24 hours after dosing, for measurement of blood glucose, plasma insulin glargine (plus M1 and M2 metabolites), and serum C-peptide.

Participants will visit the study site for 3 single-dose periods with specified washouts (7-14 days between treatment periods 1 and 2; and 7-21 days between treatment periods 2 and 3). The entire duration of the study will be a maximum of 11 weeks.

8.3. Measures to Minimize Bias: Study Treatment Assignment and Blinding

8.3.1. Method of Study Treatment Assignment

Participants will be assigned a screening identification (ID) number when providing informed consent. A randomization ID will be provided on Day 1, if the participant is still eligible. A randomization ID consists of a site ID and a participant number that uniquely identifies each participant.

On Day 1, participants will be randomly allocated to study treatment sequence by the unblinded Pharmacist according to a pre-specified randomization scheme prepared by an independent statistician (refer to Section 8.2).

8.3.2. Blinding

All participants, Investigators, and study personnel involved in the conduct of the study will be blinded to treatment assignment with the exception of unblinded monitor, statistician, programmer, pharmacists, and drug administrators.

Because study drug and reference products differ slightly in appearance, the study drug will be prepared by an unblinded pharmacist and administered by an unblinded administrator, while the participant is blindfolded. The used pen will be returned to the packaging and reconciliation will be completed by the unblinded pharmacist.

The unblinded study personnel will not participate in study procedures or data analysis prior to unblinding of the study data.

The following controls will be employed to maintain the double-blind status of the study:

- The randomization list will be kept within the Clinical Data Management System and role-based access will prevent any unauthorized personnel from accessing the blinded information. The pharmacist will have access to the randomization list for dispensing purposes.
- To manage the participant's condition in case of a medical emergency, the Investigator will be able to break the blind for a specific participant, using the Clinical Data Management System, to know whether a participant received NKF-INS(G), US-Lantus or EU-Lantus. In order to break the blind, the Investigator will have to provide a reason for doing so. All relevant data including the responsible individual, the date, the time, and the reason for breaking the blind will be captured electronically within audit trail of the Clinical Data Management System. The Sponsor will be informed in case of unblinding.
- Measures (where relevant) will be put in place to maintain the blind during dosing and will be described in the blinding plan.

8.4. Treatment Modifications and Discontinuations

Dose reductions and adjustments are not permitted; any dose reductions and adjustments that occur will be recorded and considered protocol deviations.

Delays and omissions of study treatment administration because of medical or nonmedical reasons are not permitted. All delays and omissions of study treatment that occur during the study will be recorded and considered protocol deviations.

A participant may be discontinued from the study at any time for reasons including, but not limited to, the following:

- AE and/or SAEs in the judgment of the Investigator that may prevent the participant from continuing participation in the study.
- Participant withdrawal of consent (at any time, a participant's participation in the study may be terminated at their request).
- On the basis of the Investigator's clinical judgment.

- General or specific changes in the participant's condition that render them ineligible for further treatment according to the inclusion/exclusion criteria.
- Participant fails to adhere to the protocol requirements (e.g., study treatment noncompliance).
- Lost to follow up: the participant stopped coming for visits and study personnel were unable to contact the participant.

Note: 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 make contact with 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 medical records.

Any participant who discontinues from the study will be asked to return to the clinic for an EOS visit that includes the assessments as shown in the schedule of assessments (Table 3). The reason and date for the participant's discontinuation from the study must be recorded in the eCRF.

8.5. Treatment Accountability and Compliance

The pharmacist or other designated individual will maintain records of study drug delivered to the study site, the inventory at the study site, the distribution to and use by each participant, and the return of materials to the Sponsor for storage or disposal/destruction of materials at the study site. These records must include dates, quantities, lot/batch/serial numbers, expiration dates, storage temperature log, and unique code numbers assigned to the study drug and study participants.

The pharmacist will maintain records that adequately document that the participants were provided with the correct study treatment and reconcile the products received from the Sponsor. Investigational product will not be returned to the Sponsor or disposed of until accountability has been fully monitored.

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. Discontinuation for noncompliance is at the Investigator's discretion and is to be noted in the eCRF.

Cases of accidental overdose or medication error, along with any associated AEs, should be reported as described in Section 12.6.9.

8.6. Prior and Concomitant Therapy and Other Restrictions During the Study

8.6.1. Prior and Concomitant Medications

Medications taken by or administered to the participant for the period before screening will be recorded in the eCRF as prior medications. Restricted prior 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, or thyroid replacement).

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 enrollment, or receives during the study, must be recorded in the eCRF as concomitant medications along with:

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

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

All participants will receive daily iron supplementation (e.g., QuadroFer or equivalent) starting at discharge of treatment period 1 and continuing for 60 days.

All previous and on study Coronavirus disease 2019 (COVID-19) vaccinations should be recorded in the eCRF.

The Medical Monitor should be contacted if there are any questions regarding prior or concomitant therapy.

8.6.1.1. Permitted Concomitant Medications and Therapies

Other than those medications explicitly prohibited (Section 8.6.1), concomitant medication decisions will be left to the discretion of the Investigator.

8.6.1.2. Prohibited Concomitant Medications and Therapies

Participants must be instructed not to take any medications, including over-the-counter products, without first consulting with the Investigator.

If the participant has received any other investigational drug within 3 months before the administration of study treatment, he will not be eligible for this study.

No prophylactic medication, treatments, or procedures will be administered during the study period, unless at the discretion of the Investigator.

8.6.1.3. Advisory on COVID-19 Vaccination and Risk Assessment

The COVID-19 pandemic is an ongoing pandemic with varying impact at any time in different geographies worldwide (WHO, 2024). It is recommended that participants and their investigators discuss on an individual basis the benefits and risks of COVID-19 vaccination in the context of their prior vaccination history and consider the vaccinations approved by national regulatory agencies, as well as COVID-19 vaccination guidance from scientific and medical associations.

The following recommendations should be considered for the study:

- Participants with prior COVID-19 vaccination may be allowed to enter the study with a washout period of at least 7 days since the last COVID-19 vaccine dose.

- Administration of a COVID-19 vaccine during participation in the study should be documented as a concomitant medication (Section 8.6.1).
- Previous administration(s) of COVID-19 vaccine should be documented in the prior and concomitant medication form.

8.6.2. Other Restrictions During the Study

8.6.2.1. Meals and Dietary Restrictions

For all treatment periods, participants will fast for 10-12 hours prior to dosing and until the glucose clamp procedure is completed, after which participants will receive a meal. There is no restriction on water during the fasting periods of the study. When not in the CRU, participants will be encouraged to follow their normal diets. Grapefruit juice is an inhibitor of the CYP3A4 enzyme but CYP3A4 does not play a role in the metabolism of insulin glargine thus it is not mentioned.

8.6.2.2. Caffeine, Alcohol and Tobacco

No alcohol will be allowed 48 hours before each dose and for the duration of each CRU visit.

Participants should refrain from caffeine-containing food/beverages for at least 24 hours before each dose and through the duration of visit.

Smoking of tobacco products, nicotine replacements etc., will not be permitted during the confinement period.

8.6.2.3. Activity

Participants are encouraged to maintain their regular exercise habits; however, they should not undertake vigorous or prolonged exercise within 48 hours prior to dosing. Strenuous exercise is not allowed throughout the entire study.

9. STUDY PROCEDURES

Assessments and their timing to be performed are outlined in the schedule of assessments (Table 3).

See Sections 10, 11, 12, and 13 for additional details regarding PK, PD, safety, and other assessments, respectively.

The Investigator may, at his or her discretion, arrange for a participant to have an unscheduled assessment, especially in the case of AEs that require follow up or are considered by the Investigator to be possibly related to the use of study treatment. The unscheduled visit page in the eCRF must be completed for all unscheduled assessments.

Table 3: Schedule of Assessments

Assessment	Screening	Euglycemic Clamp Procedure Treatment Periods 1-3[1]			EOS Visit
	-28 to -1 Days Prior to Treatment Period 1	Day -1	Clamp Procedure Starting the Morning of Day 1 for 24 Hours[2]	Post- Clamp (Day 2)	Within 2-11 Days After Last Administration of Study Drug
Informed consent	X				
Demographic data[3]	X				
Alcohol and tobacco consumption patterns	X				
Medical and medications history[4]	X	X			
Inclusion/exclusion criteria	X	X[5]	X[5]		
Admission to CRU[6]		X			
Randomization			X[5]		
Discharge from CRU				X	
Height/weight[7]	X	X			
Physical examination[8]	X				X
Vital signs[9]	X		X	X	X
12-lead ECG	X				X
Hematology[10]	X				X
Clinical chemistry[11]	X				X
Blood coagulation[12]	X				
Serology tests (HBsAg, IgM anti-HBc, HCV, HIV) [13]	X				
Urinalysis[14]	X				
Urine screen for drugs of abuse[15]	X	X			
Alcohol breath test[16]	X	X			
Human insulin antibodies	X				
Oral glucose tolerance test[17]	X				
Study drug administration[18]			Day 1 at time = 0 hours/0 min		
Injection site assessment[19]			X		X
GIR			X		
Adverse events			→		
Concomitant medications[20]	→				

Assessment	Screening	Euglycemic Clamp Procedure Treatment Periods 1-3[1]			EOS Visit
	-28 to -1 Days Prior to Treatment Period 1	Day -1	Clamp Procedure Starting the Morning of Day 1 for 24 Hours[2]	Post- Clamp (Day 2)	Within 2-11 Days After Last Administration of Study Drug
PK, PD, C-peptide, and glucose sampling (see Table 4)					

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; anti-HBc = antibody to hepatitis B core antigen; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; CRU = clinical research unit; ECG = electrocardiogram; eCRF = electronic case report form; EOS = end of study; GGT = gamma-glutamyl transferase; GIR = glucose infusion rate; Hb = hemoglobin; HBsAg = hepatitis B surface antigen; HCT = hematocrit; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IgM = immunoglobulin M; INR = international normalized ratio; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; PD = pharmacodynamic; PK = pharmacokinetic; PT = prothrombin time; RBC = red blood cell; WBC = white blood cell

1 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.

2 The clamp procedure will be considered as ended if the GIR is on zero (0) and the infusion pump has been stopped, or the 24-hour mark is reached, whichever is first. Blood glucose concentration will be measured for 3 consecutive times for safety reasons, after the infusion pump is on zero (0), unless the 24-hour mark is reached.

3 Sex, race, year of birth, age, and ethnicity.

4 The recorded medical history will be updated, if necessary, at admission to treatment period 1.

5 Randomization and final eligibility check will only be done in treatment period 1.

6 Participants will be admitted to the CRU on Day -1, approximately 12 hours prior to dosing.

7 Body weight will be recorded at screening and on Day -1 of all treatment periods. The weight measured on Day -1 of treatment period 1 will be used for dosage calculations of insulin for all treatment periods. Height and body mass index will be recorded at screening only.

8 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 evaluation; musculoskeletal assessment; neurological assessment; other evaluations may be performed as deemed necessary by the Investigator. A full physical exam should be done at screening. A limited symptom- based physical exam may be done at EOS.

9 Supine systolic and diastolic blood pressure, pulse, and respiratory rate will be recorded at screening, before administration of the study drug, at the end of the clamp (before discharge from the study center) and at the EOS visit. Body temperature (temporal or ear) will be recorded at screening and before administration of study drug (either standing or supine). All vital signs will be measured after the participant has been resting in a supine position for at least 5 minutes.

10 Hematology (ethylenediaminetetraacetic acid tubes): full and differential blood count, WBC count, RBC count, Hb, HCT, MCV, MCH, MCH concentration, absolute differential count (neutrophils, lymphocytes, monocytes, eosinophils and basophils), and platelets. Laboratory assessments may be repeated once during screening at the discretion of the investigator.

11 Clinical chemistry (serum separator tubes): potassium, sodium, urea, chloride, phosphorus, creatinine, calcium, albumin, total and direct bilirubin, creatine kinase, ALP, GGT, AST, ALT, lactate dehydrogenase, total cholesterol, triglycerides, and glucose. Laboratory assessments may be repeated once during screening at the discretion of the investigator.

12 Blood coagulation tests (citrated tubes): PT, aPTT, and INR. Laboratory assessments may be repeated once during screening at the discretion of the investigator.

13 Tests for HIV, HBsAg, IgM anti-HBc, and HCV will be performed using commercially available test kits. Pre- and post-test counseling will be provided as appropriate.

14 Urinalysis (dipstick): appearance, bilirubin, glucose, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrite, leucocytes. Abnormal urinalysis results may be repeated at the discretion of the Investigator.

15 A rapid, one-step screening test for simultaneous, qualitative detection of multiple drugs and drug metabolites will be used. Participants with alleged false positive test results will be excluded from the study. However, a positive test may be repeated once at the discretion of the Investigator.

16 Alcohol breath test using a portable breath alcohol measuring device. The test will be performed at screening, at admission to each treatment period and at random. If any of these tests are positive, the subject will not be allowed further participation in the study.

17 Oral glucose tolerance test may be completed after all other eligibility has been determined. For participants who re-screen, oral glucose tolerance test does not need to be repeated if completed within the last 6 months.

18 From 1 hour before study drug administration until 3 hours post-dose, the injection site will be kept at a constant temperature. The time of injection is defined as time zero (0 hour/0 min) and marks the start of the clamp period, which will continue for up to 24 hours or until the GIR is 0 and blood glucose remains stable.

19 The injection site will be assessed at 1 min and 15 min post-dose on Day 1 and at the EOS visit. Reactions to the study drug injection will be documented in the eCRF.

20 Participants will receive daily iron supplementation (e.g., QuadroFer or equivalent) starting at discharge of treatment period 1 and continuing for 60 days

Table 4: Pharmacokinetic, Pharmacodynamic, C-Peptide, and Glucose Sampling Schedule

	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 →					

9.1. Screening and Baseline Assessments

Standard study procedures will be performed at the timepoints indicated in the schedule of assessments (Table 3). A description of the study procedures is provided in the sections below.

9.1.1. Informed Consent

Before performing any study-related procedures, the Investigator (or designee) will obtain written informed consent from the participant according to national legal regulations (see Section 15.1.3).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened only once. Participants may be rescreened at the Investigator's discretion. The participant must be reconsented and a new screening number will be assigned. Also, a new ICF must be signed if updates have been made since the signing of the first ICF.

To give the participant sufficient time to consider study participation, informed consent can be obtained before the screening visit. However, if the informed consent is obtained on the day of screening, then the time must be documented in the participant's chart to provide proof that consent was given before the first study-related screening procedure.

- The Investigator or their authorized representative will explain the nature of the study to the participant and answer all questions regarding the study.
- Participants will be informed that their participation is voluntary and will be required to sign a statement of informed consent that meets the requirements of the local regulations, the ICH guidelines, the REC/IRB, or study site.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date (and time, if obtained on the day of screening) when the written consent was obtained. The ICF will also be signed by the authorized investigator obtaining the ICF or by another authorized investigator according to the FARMOVS SOP.
- Participants must give new consent to each updated version of the ICF during their participation in the study.
- Each ICF must be personally dated and signed by the participant as well as by the Investigator (or designee).
- A copy of the ICF (or second original ICF per local regulations) must be offered to the participant. If the ICF is not taken by the participant, it will be archived with the participant's study documents.

9.1.2. Demographics

Year of birth and/or age, sex at birth, race, and ethnicity will be recorded at screening in the eCRF.

9.1.3. Medical History

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.

Illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs in the eCRF in accordance with Section 12.6. All changes that are not present at baseline or described in the medical history and identified as clinically noteworthy must be recorded as AEs.

9.1.4. Eligibility Review

Participant eligibility will be reviewed per the inclusion criteria (Section 7.1) and exclusion criteria (Section 7.2) at the timepoints specified in the schedule of assessments (Table 3). Before randomization on Day 1, the eligibility review must be completed.

9.1.5. Drug and Alcohol Screening

A urine drug test for prohibited substances, using a rapid, one-step screening test for simultaneous, qualitative detection of multiple drugs and drug metabolites will be performed at the timepoints specified in the schedule of assessments (Table 3). Results must be negative for study eligibility. However, if a participant has a positive result for a medically indicated substance such as benzodiazepines, and the Investigator determines that the participant was not abusing the substance, the positive result will not be exclusionary, provided that the Investigator consults with the Medical Monitor before enrolling or randomizing/dosing the participant.

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

9.1.6. Serology Testing

Serum testing for HBsAg, IgM anti-HBc, HCV, and HIV will be performed at the timepoints specified in the schedule of assessments (Table 3).

9.1.7. Prior and Concomitant Medication

Any medication or therapy other than NKF-INS(G), US-Lantus, and EU-Lantus is considered concomitant medication (see Section 8.6.1) and should be recorded in the eCRF.

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

All previous and on study COVID-19 vaccinations should be recorded in the eCRF.

9.1.8. Other Screening Assessments

Other screening assessments include physical examination, height weight, and body mass index, vital signs, 12-lead ECG, and safety laboratory assessments.

An oral glucose tolerance test will be conducted per site SOP. Oral glucose tolerance test may be completed after all other eligibility has been determined. For participants who re-screen, oral glucose tolerance test does not need to be repeated if completed within the last 6 months.

The participants will also be screened for human insulin antibodies.

These assessments are also conducted at other timepoints as detailed in the schedule of assessments (Table 3).

10. PHARMACOKINETICS

10.1. Pharmacokinetic Sampling

10.1.1. Blood Samples

Blood samples for PK analysis of NKF-INS(G), US-Lantus, and EU-Lantus will be collected at the timepoints indicated in Table 4. The actual date and time of each blood sample collection will be recorded. Actual time will be used for derivation of PK parameters.

Details of PK blood sample collection, processing, storage, and shipping procedures are provided in a separate laboratory manual.

10.1.2. Pharmacokinetic Evaluation

The PK parameters will be derived for NKF-INS(G), US-Lantus, and EU-Lantus from plasma concentrations by a validated PK analysis platform using standard noncompartmental methods (where data allow). A description of the PK parameters is provided in Table 5. A complete list of PK parameters will be provided in the statistical analysis plan (SAP).

Table 5: Pharmacokinetic Parameters

PK Parameter	Description
$AUC_{0\text{-}12h}$	concentration-time curve from 0 to 12 hours
$AUC_{0\text{-}6h}$	concentration-time curve from 0 to 6 hours
$AUC_{0\text{-}\infty}$	concentration-time curve from 0 hour to infinity
$AUC_{0\text{-}24h}$	concentration-time curve from 0 hour to 24 hours
$AUC_{12\text{-}18h}$	concentration-time curve from 12 to 18 hours
$AUC_{12\text{-}24h}$	concentration-time curve from 12 to 24 hours
$AUC_{18\text{-}24h}$	concentration-time curve from 18 to 24 hours
$AUC_{6\text{-}12h}$	concentration-time curve from 6 to 12 hours
C_{max}	maximum observed concentration
M1	21A-Gly-human insulin
M2	21A-Gly-des-30B-Thr-human insulin
T_{max}	time to maximum plasma concentration

If terminal phase of plasma concentration-time profile has sufficient data points, apparent terminal elimination half-life (t½.z), apparent terminal elimination rate constant (λz), mean residence time (MRT), total body clearance (CL/F), and volume of distribution (V/F) will be calculated.

PK parameters will be estimated using noncompartmental methods. Estimates of PK parameters will be listed and summarized (N, arithmetic and geometric mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) by treatment group), descriptively and graphically.

The full analysis will be described in the prospective SAP that will be finalized prior to database lock.

10.1.3. Pharmacokinetic Analytical Methodology

The concentration of insulin glargine, M1, and M2 following administration of NKF-INS(G), US-Lantus, and EU-Lantus will be determined from the plasma samples using a validated analytical method. Details of the method validation and sample analysis will be included with the final clinical study report.

11. PHARMACODYNAMICS

11.1. Pharmacodynamic Sampling

11.1.1. Blood Samples

Blood samples following administration of NKF-INS(G), US-Lantus, and EU-Lantus will be collected at the timepoints indicated in the sample schedule shown in Table 4. The actual date and time of each blood sample collection will be recorded. Actual time will be used for derivation of PD parameters.

Details of PD blood sample collection, processing, storage, and shipping procedures are provided in a separate laboratory manual.

11.1.2. Pharmacodynamic Evaluation

The following PD parameters will be derived for NKF-INS(G), US-Lantus, and EU-Lantus by a validated PK analysis program using standard noncompartmental methods (where data allow):

The PD similarity will be assessed using the parameters provided in Table 6.

A complete list of PD parameters will be provided in the SAP.

Table 6: Pharmacodynamic Parameters

PD Parameter	Description
$GIRAUC_{0\text{-}6h}$	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
$GIRAUC_{0\text{-}24h}$	GIRAUC from 0 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
$TGIR_{max}$	time to maximum GIR

Abbreviation: GIR = glucose infusion rate

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 graphically.

Endogenous insulin suppression via C-peptide measurements will also be explored.

11.1.3. Pharmacodynamic Analytical Methodology

The PD parameters listed in Table 6 will be determined from whole blood glucose collection using a validated analytical method. The concentration of C-peptide following NKF-INS(G), US-Lantus, and EU-Lantus will be determined from the serum samples using a validated analytical method. Details of method validation and sample analysis will be included with the final clinical study report.

12. SAFETY ASSESSMENTS

Safety assessments (vital signs, physical examinations, ECG recordings, clinical laboratory assessments [hematology, clinical chemistry (including glucose), coagulation, and urinalysis], and AE monitoring [including injection site reactions]) are to be performed as outlined in the schedule of assessments (Table 3).

12.1. Vital Signs

Vital signs (heart rate, respiratory rate, systolic and diastolic blood pressure, and temporal or ear temperature measurements) will be evaluated at the timepoints indicated in the schedule of assessments (Table 3). All vital signs will be measured after the participant has been resting in a supine position for at least 5 minutes. Height (without shoes) will be recorded at screening. Weight will be recorded at screening and on Day -1 of each treatment period.

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 will be repeated at the Investigator's discretion. Any confirmed, clinically significant abnormal vital sign measurements must be recorded as AEs.

12.2. Physical Examination

A full physical examination will be performed at screening. 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 evaluation; musculoskeletal assessment; neurological assessment. Other evaluations may be performed as deemed necessary by the Investigator.

A limited or symptom-driven physical examination to follow up regarding any change in medical history will be performed at EOS as indicated in the schedule of assessments (Table 3) or at the Investigator's discretion. Symptom-driven, limited physical examinations may be performed as clinically indicated or at the discretion of the Investigator at any study visit. Clinically significant findings must be recorded as AEs.

12.3. Electrocardiograms

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

At screening, the Investigator will examine the ECG traces for signs of cardiac disease that could exclude the participant from the study. An assessment of normal, 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 will be repeated if clinically significant abnormalities are observed, or artifacts are present. Any new or worsening results that are confirmed, clinically significant abnormal ECG findings after screening must be recorded as AEs.

12.4. Laboratory Assessments

Laboratory assessment samples (Table 7) are to be obtained at designated timepoints as detailed in the schedule of assessments (Table 3). Laboratory assessments may be repeated once during screening at the discretion of the investigator.

Table 7: Laboratory Assessments

Hematology:	**Clinical Chemistry:**	**Urinalysis (Dipstick):**
Full and differential blood count Hematocrit Hemoglobin Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Mean corpuscular volume Platelet count Red blood cell count White blood cell count with Differential (absolute values): Basophils Eosinophils Lymphocytes Monocytes Neutrophils	Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bilirubin (direct and total) Urea Creatine kinase* Creatinine Electrolytes (calcium, chloride, phosphorus, potassium, sodium) Gamma-glutamyl transferase Glucose Lactate dehydrogenase Total cholesterol Triglycerides Oral glucose tolerance test	Appearance Glucose Blood Leucocytes Ketone bodies pH Protein Specific gravity Urobilinogen Bilirubin Nitrite
Coagulation:	**Other:**	**Serology:**
PT aPTT INR	Urine drug Human insulin antibodies	HBsAg IgM anti-HBc HCV HIV

Abbreviations: anti-HBc = hepatitis B core antigen; aPTT = activated partial thromboplastin time; CK-MB = creatine kinase MB; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV = human immunodeficiency virus; IgM = immunoglobulin M; INR = international normalized ratio; PT = prothrombin time.
*In case of elevated creatine kinase, CK-MB may be performed, at the discretion of the Investigator

The tests detailed in Table 7 will be performed. All laboratory reports must be reviewed, signed, and dated by the Investigator. A legible copy of all reports must be filed with the participant's medical record (source document) for that visit. 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.

Further laboratory assessments outside the protocol-defined schedule may be obtained at the discretion of the Investigator and used for other clinical treatment decisions.

12.5. 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. A global irritation score, other clinical observations, and superficial observations will be assessed and documented in the eCRF.

12.6. Adverse Events

12.6.1. Definitions

12.6.1.1. Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical investigation participant administered an investigational product which does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product.

12.6.1.2. Adverse Reaction

A response to a medicine in humans which is noxious and unintended, and which occurs at any dose, and which can also result from overdose, misuse, or abuse of a medicine. Response to a medicinal product in this context means that a causal relationship between the medicinal product and an AE is at least a reasonable possibility.

12.6.1.3. Device Reportable Adverse Event

Any event associated with an NKF-INS(G) BD Vystra Pre-filled Pen (study product pen), US-Lantus SoloStar (reference product pen) or EU-Lantus SoloStar (reference product pen) that meets three basic reporting criteria, even if it does not involve a patient or user, should be reported to the Regulatory Authority if:

- An adverse event has occurred
- The licensed manufacturer’s or licensed distributor’s medical device is associated with the adverse event
- The event led to or might lead to (often referred to as a near adverse event) death or serious injury*, or might lead to death or serious injury if it were to occur again.

* Serious Injury includes:

- A life-threatening illness or injury
- Permanent impairment of a body function
- Permanent damage to a body structure
- A condition necessitating medical or surgical intervention to prevent permanent impairment of a body function or permanent damage to a body structure

12.6.1.4. Serious Adverse Event

An SAE is defined as any untoward medical occurrence, in the view of either the Investigator or Sponsor, 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 an overnight stay. Complications occurring during hospitalization are AEs and SAEs if they cause prolongation of the current hospitalization. Hospitalization for elective treatment of a preexisting, non-worsening condition 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

In addition, medical and scientific judgment is required to decide if prompt notification is required in situations other than those defined for SAEs. This may include any event that the Investigator regards as serious that did not strictly meet the criteria above but may have jeopardized the participant or required intervention to prevent one of the outcomes listed above, or that would suggest any significant hazard, contraindication, AE, or precaution that may be associated with the use of the study treatment.

Other important medical events that may not be immediately life-threatening or result in death or hospitalization, based upon appropriate medical judgment, 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. SAEs are critically important for the identification of significant safety problems; therefore, it is important to consider both the Investigator's and the Sponsor's assessment.

12.6.1.5. Treatment-Emergent Adverse Event

An AE with a start date and time on or after the first dose of study drug.

12.6.1.6. Unexpected Adverse Reaction

An adverse reaction in which the nature, specificity, severity, and outcome is not consistent with the referenced safety information.

12.6.2. Adverse Event Collection, Assessment and Reporting

12.6.2.1. Observation Period for Assessment of Adverse Events

For the purposes of this study, the period of observation for collection of AEs (including nonserious AEs and SAEs) extends from the time the participant begins study treatment (Day 1) until the EOS visit (within 2-11 days after last administration of the study drug). If a participant withdraws or is discontinued from the study by the Investigator, AEs should be collected from the time the participant begins study treatment (Day 1) up to the completion of EOS assessments.

12.6.2.2. Parameters for Adverse Events Collection

All nonserious AEs and SAEs will be recorded on the AE eCRF. SAEs will also be immediately reported to the Sponsor safety representative in accordance with Section 12.6.5.

Nonserious and SAEs will be documented in the clinical database with the highest severity documented during the duration of each serious or nonserious episode. Each AE is to be documented in the eCRF with reference to date of onset, severity, relationship to study drug, treatment of event, and outcome.

All AEs are to be followed up until resolution or a stable clinical endpoint is reached, as determined by the Investigator. Changes in AEs and resolution dates are to be documented in the eCRF.

Participants will be instructed by the Investigator to report AEs at each study visit. All volunteered, elicited, and observed AEs are to be recorded. A consistent methodology of nondirective questioning should be adopted for eliciting AE information at all participant evaluation timepoints. Examples of nondirective questions include the following:

- "How are you feeling?"
- "How have you felt since your last clinic visit?"
- "Have you had any new or changed health problems since you were last here?"
- "Have you taken any new medications since your last visit?"

The following considerations should also be made regarding AE and SAE reporting:

- All medical and psychiatric conditions present at screening (i.e., conditions present before the AE reporting period) will be considered preexisting conditions and documented in the medical history eCRF. A preexisting condition is not to be reported as an AE. If a preexisting condition worsens during the study treatment period, the event should be reported as an AE. Planned hospitalization for a preexisting condition, or a procedure required by the protocol, without serious deterioration in health, is not considered an SAE.
- Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. A medical condition for which an unscheduled procedure was performed, should however be reported if it meets the definition of an AE. For example, acute appendicitis, and not the appendectomy, should be reported as the AE.
- Clinically significant abnormal laboratory findings (e.g., hematology, clinical chemistry [including glucose], coagulation, and urinalysis) or other abnormal assessments (e.g., in ECG results and vital signs), meeting the definition of an AE or SAE, that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. The Investigator will exercise his/her medical and scientific judgment in deciding whether an abnormal laboratory finding, or other abnormal assessment is clinically significant.

Special situations are non-standard medical conditions that provide valuable information (e.g., clinical, safety) about a medicinal product, even when special situations do not occur in association with an AE or medical condition. Special situations, defined in this protocol as events of accidental overdose, medication error, and drug misuse, are not in and of

themselves AEs, but may result in AEs. Each AE associated with a special situation should be recorded in the AE eCRF. If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

In the event of an accidental overdose, medication error, or drug misuse special situation, the Investigator should:

- Inform the Medical Monitor immediately.
- Closely monitor the participant for an AE/SAE and laboratory abnormalities.
- In the event of an accidental overdose: document the quantity of excess dose in the eCRF.

12.6.3. Assessment of Event Severity

The Investigator will use the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (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.

12.6.4. Relationship to Study Treatment

Specific guidelines for classifying AEs by relationship to study treatment are given in Table 8.

Table 8: Classification of Adverse Events 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 occur 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.

12.6.5. Serious Adverse Event Reporting

Any SAE occurring during the course of the study within the defined AE reporting window, must be immediately reported to the appropriate Sponsor safety representative (PrimeVigilance) within 1 calendar day (i.e., within 24 hours) of the investigational site's initial awareness of the event. The study Safety Report Form, provided by the Sponsor, will be utilized for reporting safety information to the Sponsor safety representative and completed in English. For fatal or life-threatening treatment-emergent SAEs, where important or relevant information is missing, active follow up will be undertaken immediately by the Investigator.

A post-study AE or SAE is defined as any event that occurs outside of the AE/SAE monitoring period. Investigators are not obligated to actively monitor AEs or SAEs of such study participants. However, if the Investigator learns of any AE or SAE, including a death, at any time after a participant has completed the study, and it is considered reasonably related to the study treatment, the Investigator should promptly notify the Sponsor safety representative within 24 hours of becoming aware of the event.

Investigators or other site personnel will inform the Sponsor safety representative of any follow-up information on a previously reported SAE within 1 calendar day (i.e., within 24 hours) of the investigational site's awareness of the new information. All SAEs will be followed until satisfactory resolution, until the Investigator deems the event to be chronic, or the participant is stable. Other supporting documentation of the event may be requested by the Sponsor or Sponsor safety representative and should be provided by the Investigator as soon as possible.

The Sponsor will notify regulatory authorities and participating Investigators of any reportable SAEs for NKF-INS(G), US-Lantus, and EU-Lantus) in an expedited fashion in accordance with local requirements. Where required and in accordance with local requirements, the Sponsor will ensure unblinding of subject treatment assignment for the purposes of reporting the event to regulatory authorities. IRBs/RECs will be promptly notified, in accordance with local regulations, of all reportable SAEs involving risk to human participants. Where reportable SAEs are identified for referenced products, i.e., US-Lantus and EU-Lantus, the Sponsor will ensure the product manufacturer is notified within the same expedited timelines as regulatory authorities.

For further guidance on the definition of an SAE, see Section 12.6.1.4.

12.6.6. Insulin Pen Adverse Event Reporting

Any AE, occurring during the course of the study within the defined AE reporting window, that is considered related to an insulin pen, must be immediately reported to the appropriate Sponsor safety representative (PrimeVigilance) within 1 calendar day (i.e., within 24 hours) of the investigational site's initial awareness of the event. The study Safety Report Form, provided by the Sponsor, will be utilized for reporting safety information to the Sponsor safety representative and completed in English.

Investigators or other site personnel will inform the Sponsor safety representative of any follow-up information on a previously reported insulin pen related AE within 1 calendar day (i.e., within 24 hours) of the investigational site's awareness of the new information. All insulin pen related AEs will be followed until satisfactory resolution, until the Investigator deems the event to be chronic, or the participant is stable. Other supporting documentation of the event may be requested by the Sponsor or Sponsor safety representative and should be provided by the Investigator as soon as possible.

The Sponsor will notify regulatory authorities in an expedited fashion of any device reportable AE complaints associated with the BD Vystra disposable pen, US-Lantus SoloStar pen, or EU-Lantus SoloStar pen in accordance with local requirements. For further guidance on the definition of a device reportable AE, see Section 12.6.1.3.

12.6.7. Drug-Induced Liver Injury Monitoring

For new elevations in transaminases greater than 2 × the upper limit of normal (ULN) in participants with normal baseline values, repeat measurement will be performed within 48 hours. If elevations persist, participants will be evaluated for other causes of transaminase elevations and with tests of hepatic function. If no other cause is found, then the participants will be monitored closely and the drug discontinued as per the recommendations in the FDA Guidance for Industry – Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA, 2009).

The participant should be followed until resolution of symptoms or signs in the following situations:

- Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >8 × ULN.
- ALT or AST >5 × ULN for more than 4 days during the 7-day treatment period.
- ALT or AST >3 × ULN (and total bilirubin >2 × ULN or international normalized ratio [INR] >1.5).
- ALT or AST >3 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%).

12.6.8. Pregnancy

After administration of study treatment, any known cases of pregnancy in female partners of male study participants will be reported until the male participant completes or withdraws from the study and within 90 days of their exposure to study treatment. The Investigator may arrange counseling for pregnant female partners of male study participants by a specialist to discuss the risks of continuing with the pregnancy and the possible effects on the fetus. The pregnancy will be reported immediately by faxing/emailing a completed pregnancy report to the Sponsor safety representative (or designee) within 24 hours of knowledge of the event. The pregnancy will not be processed as an AE/SAE; however, the Investigator must follow up with female partners of male study participants until completion of the pregnancy and must assess the outcome in the shortest possible time, but not more than 30 days, after completion of the pregnancy. The Investigator should notify the Sponsor (or designee) of the pregnancy outcome by submitting a follow-up pregnancy report. If the outcome of the pregnancy involved spontaneous or therapeutic abortion (any congenital anomaly detected in an aborted fetus is to be documented as an SAE), stillbirth, neonatal death, or congenital anomaly, the Investigator will report the SAE by faxing/emailing a completed pregnancy report form to the Sponsor safety representative (or designee) within 24 hours of knowledge of the event. Any pregnancy in female partners of male study participants will be followed to term, and the status of the mother and child will be reported to the Sponsor after delivery.

Contraception guidance is provided in Appendix 1.

12.6.9. Accidental Overdose, Medication Error, or Drug Misuse

Accidental overdose, medication error, and drug misuse, hereafter collectively referred to as "special situations," are defined as follows:

- Accidental overdose: accidental administration of a drug in a quantity that is higher than the assigned dose.
- Medication error: accidental deviation in the administration of a drug. In some cases, a medication error may be intercepted before administration of the drug.
- Drug misuse: intentional deviation in the administration of a drug that does not qualify as drug abuse.

Cases of accidental overdose, drug misuse, or medication error without manifested signs or symptoms are not considered AEs. AEs associated with a special situation will be documented according to Section 12.6.2.2. SAEs associated with a special situation should be reported according to the procedure outlined in Section 12.6.5. All special situations (with or without associated AEs) will be documented on the eCRF.

13. OTHER ASSESSMENTS

13.1. Euglycemic Clamp Procedure

An euglycemic glucose clamp at targeted blood glucose concentrations will be utilized after administration of NKF-INS(G), US-Lantus, and EU-Lantus. The euglycemic glucose clamp will be conducted using a manual clamp technique, which will continuously monitor the subject's blood glucose and administer 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 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 dosing and until end of clamp, up to 24 hours after dosing, for measurement of blood glucose, plasma insulin glargine (plus M1 and M2 metabolites), and serum C-peptide as shown in the schedule of assessments (Table 3).

14. STATISTICAL ANALYSIS

A SAP will be prepared after the protocol is approved. The SAP will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives. The SAP will serve as a complement to the protocol and supersedes it in case of differences.

The statistical evaluation will be performed using R version 4.0 or higher. All data will be listed, and summary tables and figures will be provided. Summary statistics will be presented by treatment group, to the extent possible. For continuous variables, data will be summarized with the number of participants (N), arithmetic mean, SD, median, minimum, and maximum by treatment group. Geometric mean and CV% will be presented as appropriate, by treatment group. For categorical variables, data will be tabulated with the number and proportion of participants for each category by treatment group.

A fixed sequence method will be used to control for multiplicity of the endpoint assessments. The endpoints are ordered as follows:

Primary Endpoint:

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

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

- $GIRAUC_{0\text{-}24h}$
- $AUC_{0\text{-}12h}$
- $GIRAUC_{0\text{-}12h}$
- $AUC_{12\text{-}24h}$
- $GIRAUC_{12\text{-}24h}$
- $AUC_{0\text{-}\infty}$
- $GIRAUC_{0\text{-}\infty}$
- $AUC_{0\text{-}6h}$
- $GIRAUC_{0\text{-}6h}$
- $AUC_{6\text{-}12h}$
- $GIRAUC_{6\text{-}12h}$
- $AUC_{12\text{-}18h}$
- $GIRAUC_{12\text{-}18h}$
- $AUC_{18\text{-}24h}$
- $GIRAUC_{18\text{-}24h}$
- GIR_{max}
- $TGIR_{max}$
- C_{max}
- T_{max}
- $AUC_{0\text{-}24h}$ 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.

14.1. Determination of Sample Size

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 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 (Bhatia, 2021; Chen, 2023; Linnebjerg, 2015; Health Canada, 2022).

Based on the above data, a CV% of 43 was selected. Considering a 95% confidence interval and 15% drop out rate, 114 participants are planned to be enrolled to have at least 96 evaluable participants.

14.2. Analysis Populations

The following analysis populations are planned for this study:

Screening Population

The Screening Population will include all individuals who provide informed consent and provide demographic and/or baseline screening assessments, regardless of the participant's randomization status.

Enrolled Population

The Enrolled Population will include all individuals who sign the ICF.

Safety Population

The Safety Population will include all participants who receive any amount of study treatment.

Pharmacokinetic Concentration Population

The PK Concentration Population will include all participants in the Safety Population who received any amount of study treatment and have at least 1 post-baseline reportable PK result without any major protocol deviations.

Pharmacokinetic Parameter Population

The PK Parameter Population will include all participants in the PK Concentration Population who have at least 1 PK parameter derived without any major protocol deviations.

Pharmacodynamic Parameter Population

The PD Parameter Population will include all participants in the Safety Population who have at least 1 PD parameter derived without any major protocol deviations.

14.3. Pharmacokinetic Analysis

The primary PK objective is to compare the PK of NKF-INS(G) to US-approved and EU-authorized Lantus to demonstrate PK similarity. The secondary PK objective is to evaluate additional PK parameters of NKF-INS(G) compared to US-approved and EU-authorized Lantus. Details on the endpoints are provided in Table 1.

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

Biosimilarity between the test product and US-approved and EU-authorized Lantus 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 the 90% confidence intervals (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% to 125%. The test product will be compared to the reference product by means of statistical analysis with respect to the primary PK parameters using an analysis of variance after logarithmic transformation of the data. Point estimates and 90% CIs for the "test/reference" geometric mean ratios of these parameters will be provided. The primary PK parameter that will be used for the bioequivalence assessment is $AUC_{0\text{-}24h}$ of M1. All other PK-related assessments of equivalence will be considered secondary.

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

All PK analyses will be based on the PK Parameter Population. Refer to the SAP for full details.

14.4. Pharmacodynamic Analysis

The PD objective is to compare the PD of NKF-INS(G) to US-approved and EU-authorized Lantus by examining GIR profiles after a single SC dose. The secondary PD objective is to evaluate the additional parameters NKF-INS(G) compared to US-approved and EU-authorized Lantus. Details on the endpoints are provided in Table 1. PD concentrations and parameter data will be listed and summarized descriptively (N, arithmetic and geometric mean, SD, CV%, median, minimum, and maximum) by treatment group and graphically.

Consistent with PK biosimilarity, PD biosimilarity between the test product and the US-approved and EU-authorized Lantus 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 the 90% CIs for estimates of the geometric mean ratios between the PD parameters of the test and reference products in relation to the conventional bioequivalence range of 80% to 125%. The test product will be compared to the reference product by means of statistical analysis with respect to the PD parameters using an analysis of variance after logarithmic transformation of the data. Point estimates and 90% and 95% 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 $AUCGIR_{0\text{-}t}$ and will be the first secondary endpoint assessed in the fixed sequence method.

All PD analyses will be based on the PD Parameter Population. Refer to the SAP for full details.

14.5. Safety Analysis

All analyses of safety will be conducted on the Safety Population unless otherwise specified in the SAP.

All reported AEs will be graded using NCI CTCAE version 5.0 and will be coded using the Medical Dictionary for Regulatory Activities, version 26.0. The incidence of treatment-

emergent adverse events (TEAEs) will be included in incidence tables. Events with missing onset dates will be included as treatment-emergent. If a participant experiences more than 1 occurrence of the same AE, the occurrence with the greatest severity and the closest association with the study treatment will be used in the summary tables. All TEAEs will be summarized by treatment group (NKF-INS(G), US-Lantus, and EU-Lantus) as follows:

- All AEs
- Grade ≥3 AEs
- AEs related to study treatment, including related Grade ≥3 AEs
- All adverse reactions
- SAEs

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

Clinical laboratory data (hematology, clinical chemistry [including glucose], coagulation, and urinalysis) and vital signs (heart rate, respiratory rate, systolic and diastolic blood pressure, and temporal or ear temperature measurements) will be summarized by treatment group and scheduled visits using descriptive statistics, including mean values and mean change from screening values, as well as numbers of participants with values outside limits of the normal range at each timepoint. Shift tables will be provided presenting change from screening values to the EOS visit.

The frequency of ECG abnormalities (e.g., heart rate, PR interval, QRS complex, and QT-interval corrected by Fridericia's formula [QTcF]) will be summarized by treatment group and scheduled visits using counts and percentages.

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.

Injection site reactions will be summarized by treatment group and scheduled visits. The number and percentage of participants having reactions at each assessment timepoint, overall and by specific reaction subtype, will be presented.

Summary tables will be provided for concomitant medications initiated during the study period.

Clinically significant changes in physical examination findings and laboratory abnormalities will be listed as AEs.

14.6. Other Analyses

All other safety variables will be listed.

14.7. Interim Analysis

No interim analysis is planned.

14.8. Data Monitoring Committee

Not applicable.

15. STUDY MANAGEMENT

15.1. Approval and Consent

15.1.1. Compliance with Laws and Regulations

This study will be conducted in full conformance with the ICH E6 guideline for GCP and the principles of the Declaration of Helsinki or all relevant laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting) and the principles of GCP, as defined by the South African Health Products Regulatory Authority (SAHPRA) South African Guidelines for Good Clinical Practice in the Conduct of Clinical Trials (SA GCP 2020) and Good Pharmacy Practice in South Africa.

15.1.2. Institutional Review Board/Research Ethics Committee

This protocol, protocol amendments (if applicable), Investigator's Brochure, the ICFs, recruitment material and participant information sheets, any information to be given to the participant, and relevant supporting information must be submitted to the REC/IRB by the Principal Investigator (PI) and reviewed and approved by the REC/IRB before the study is initiated; no participant may undergo any procedure for determining eligibility for this study until REC/IRB approval is obtained.

The PI is responsible for providing written summaries of the status of the study to the REC/IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the REC/IRB. Investigators are also responsible for promptly informing the REC/IRB of any protocol amendments. The PI must receive approval/acknowledgement from the REC/IRB before implementation of the amendment.

In addition to the requirements for reporting all AEs to the Sponsor, Investigators must comply with requirements for reporting SAEs to the local health authority and REC/IRB.

Investigators may receive written investigational new drug safety reports or other safety-related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their REC/IRB and archived in the site's study file.

15.1.3. Informed Consent Process

15.1.3.1. Consent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required before protocol-specific procedures are performed.

15.1.3.2. Consent Procedures and Documentation

Informed consent is a process that is initiated before the participant's agreeing to participate in the study and continues throughout the study participation. Consent forms will be REC/IRB -approved, and the participant will be asked to read and review the document. The Investigator will explain the research study to the participant and answer any questions that

may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions before signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it before agreeing to participate. The participant will sign the informed consent document before any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.2. Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated at any time by the Sponsor, REC/IRB and/or regulatory authorities if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the Sponsor to Investigators, the IRBs/RECs and regulatory authorities, as well as to any CROs used in the study and provide the reason for termination. If the study is prematurely terminated or suspended, the PI will promptly inform study participants and the IRBs/RECs. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

15.3. Confidentiality and Privacy

Participant confidentiality and privacy are strictly held in trust by the participating Investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the Sponsor, representatives of the REC/IRB, regulatory agencies may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participants' contact information will be securely stored at each CRU for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or Sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the data management company responsible for data management, analysis, and reporting. This will not include the participants' contact or identifying information. Rather, individual participants and their research data will be identified by a unique study ID number. The study data entry and study management systems

used by CRUs and by data management research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived by the Sponsor.

All information generated in this study must be considered highly confidential and must not be disclosed to any people not directly concerned with the study without written prior permission from the Sponsor. Authorized regulatory officials and Sponsor personnel (or their representatives) will be allowed full access to inspect and copy the records. All study investigational products, participant bodily fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor. Participants will only be identified by unique participant numbers in eCRFs.

Every participant will be offered a copy of each version of the ICF that he signs before and during the study. Each ICF may also include authorization allowing the institution, Investigator, and Sponsor to use and disclose personal health information in compliance with the Health Insurance Portability and Accountability Act of 1996 and applicable South African laws.

15.4. Future Use of Stored Specimens and Data

No future use of stored specimens is planned.

Once samples have been analyzed, specimens will be destroyed. If no analyses have been completed within 5 years following EOS, samples will be destroyed.

15.5. Safety Oversight

Safety oversight will be under the direction of the Medical Monitor, Xentria's Medical Lead, as outlined in the Safety Management Plan and Medical Monitoring Plan.

15.6. Clinical Monitoring

CRU monitoring is conducted to ensure the participant's rights, safety and well-being, and the reliability of the trial results as the trial progresses. Study monitors will perform ongoing site monitoring (on-site or remote visits to include source data review and verification to confirm that the reported study data are accurate, complete, and verifiable from source documents, and that the conduct of the study is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

Monitoring for this study will be performed by a Sponsor-contracted CRO.

Details of clinical monitoring are documented in a Clinical Management Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

Following written SOPs, the monitors will verify that the clinical study is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices, Good Manufacturing Practices).

15.7. Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

The investigational site will provide direct access to all study-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities.

If a regulatory authority or health authority informs the investigator that it intends to conduct an inspection the Investigator shall notify the Sponsor immediately.

Independent audits may be conducted by a Sponsor-designated, qualified person to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

15.8. Data Handling and Record Keeping

15.8.1. Data Collection Responsibilities

Data collection is the responsibility of the clinical study staff at the CRU under the supervision of the site Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the eCRF derived from source documents should be consistent with the data recorded in the source documents.

Clinical data (including AEs, concomitant medications, and adverse reactions data) and clinical laboratory data will be entered into a 21 Code of Federal Regulations (CFR) Part 11-compliant data capture system provided by the CRO. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents as outlined in the Case Report Form Completion Guidelines.

15.8.2. Study Records Retention

The Investigator shall retain and preserve a copy of all records and data generated in the course of the study, specifically those defined by ICH GCP E6 as essential until:

- A minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the study drug.

These documents should be retained for a longer period, however, if required by local regulations or requested by the Sponsor. No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

At the end of the retention period, the Investigator shall notify the Sponsor in writing of her/his intent to destroy such material. The Sponsor shall have 30 days to respond to the Investigator's notice and shall have further opportunity to retain such materials.

15.9. Protocol Amendment and Protocol Deviation

15.9.1. Protocol Amendment

This protocol cannot be altered or changed except through a protocol Amendment issued by the Sponsor.

Amendments to the protocol that entail corrections of typographical errors, clarifications of confusing wording, changes in study personnel, and minor modifications that have no effect on the safety of participants, or the conduct of the study will be classed as administrative amendments. The Sponsor will ensure that acknowledgement of the amendment is received, filed, and submitted according to local regulations.

Amendments that are classed as substantial amendments must be submitted by the PI to the appropriate regulatory authorities and the RECs/IRBs for approval, and will not be implemented at sites until such approvals are received other than in the case of an urgent safety measure.

15.9.2. Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, ICH GCP, or applicable SOPs and site manuals. In addition, the study will be conducted in accordance with the applicable regulatory requirements of the country where the study is being conducted. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site Investigator to use continuous vigilance to identify and report deviations as soon as possible. All deviations must be addressed in study source documents and must be sent to the reviewing IRB/REC, per their SOPs. The site Investigator is responsible for knowing and adhering to the reviewing IRB/REC requirements. Further details about the handling of protocol deviations will be included in the Protocol Deviation Guidance Document.

The study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the participant requires immediate intervention, based on the judgment of the Investigator (or a responsible, appropriately trained professional designated by the Investigator). In the event of a significant deviation from the protocol due to an emergency, accident, or mistake, the Investigator (or designee) must contact the Medical Monitor and the Sponsor at the earliest possible time by telephone. This will allow an early joint decision regarding the participant’s continuation in the study. This decision will be documented by the Investigator and the Medical Monitor.

15.10. Ethical Considerations

This study will be conducted in accordance with this protocol, the accepted version of the Declaration of Helsinki and/or all relevant federal regulations, as set forth in Parts 50, 56, 312, Subpart D, of Title 21 of the CFR and EU 536/2014, Annex 1, D, 17 (a); and in compliance with ICH GCP guidelines, as well as local regulatory requirements in countries/regions where the study is being conducted.

RECs/IRBs will review and approve this protocol and the ICF. All participants are required to give written informed consent before participation in the study.

15.11. Financing and Insurance

Details on finance and insurance will be provided in a separate agreement between the Investigator and the Sponsor.

15.12. Publication Policy

This study will be registered at ClinicalTrials.gov, and results information from this study will be submitted to ClinicalTrials.gov and local registries as required by local regulation.

Any publication proposed to be published by Investigator shall be provided to Sponsor prior to publication or other disclosure**.** Due regard shall be given to, and adjustments to publication content and timing shall be made, based upon Sponsor's legitimate interests in publication review, e.g., ensuring compliance with the statistical analyses for the primary endpoint of the study according to, and consistent with, the Protocol; identifying any inaccuracies or errors in data analyses; obtaining patent protection; coordinating and maintaining the proprietary nature of submissions to health authorities; and protection of Confidential Information. In order to ensure that all publications related to Sponsor studies are responsible, ethical, timely, legally compliant, and scientifically fair and balanced, all publications contemplated hereunder will follow prevailing industry guidelines for good publication practices, including, but not limited to guidelines published by the International Committee of Medical Journal Editors and the position statements and guidelines published by the International Society for Medical Publication Professionals regarding good publication practices.

In the event that Institution or Investigator proposes to publish or otherwise disclose (whether orally or in writing) any findings, data, or results regarding the study, a draft thereof shall be submitted to Sponsor at least forty-five (45) days prior to submission for publication or other disclosure. Procedures set forth shall apply to publication of research and data derived from the study even when the study is terminated early or suspended for any reason. Results from the study shall not be made available to any third party by the Institution, Investigator, or any Study Site Team Participant outside of the publication procedures set out above.

15.13. Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual, perceived, or potential conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have an actual, perceived, or potential conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership has established policies and procedures for all study group members to disclose all actual, perceived, or potential conflicts of interest and will establish a mechanism for the management of all reported conflicts of interest.

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17. APPENDICES

Appendix 1 describes the contraception definitions and requirements applicable for this study.

Appendix 1. Contraception Definitions and Requirements

Definitions (CTFG, 2014)

Male participants who can father a child with a female partner of childbearing potential must agree to use birth control (2 highly effective methods of contraception including 1 barrier method) during the study period and 90 days after administration of study treatment. Male participants must refrain from donating or banking sperm for 90 days after administration of study treatment. Additionally, female partners of male participants should use highly effective contraception.

A man is considered fertile after puberty unless permanently sterile by bilateral vasectomy with documented aspermia or a bilateral orchiectomy.

Highly Effective Methods of Contraception

Highly effective methods of contraception are those that have a failure rate of <1% (when implemented consistently and correctly) and include:

- Combined (containing estrogen and progestogen) hormonal contraception associated with inhibition of ovulation (administration may be oral, intravaginal, or transdermal) initiated at least 3 months before the first dose of study treatment; and used in conjunction with a barrier method (e.g., male condom).
- Progestogen-only hormonal contraception associated with inhibition of ovulation (administration may be oral, injectable, or implantable) initiated at least 3 months before the first dose of study treatment; and used in conjunction with a barrier method (e.g., male condom).
- Intrauterine device.
- Intrauterine hormone-releasing system.
- Bilateral tubal ligation
- Vasectomy (provided that the male participant has received medical assessment of surgical success [i.e., azoospermia]).
- Sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment[s]); the reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the participant initiated at least 1 month before the first dose of study treatment.

Acceptable Methods of Contraception That Are Not Considered Highly Effective

Acceptable methods of contraception that result in a failure rate of >1% per year include:

- Progestogen-only hormonal contraception, where inhibition of ovulation is not the primary mode of action.
- Male or female condom with or without spermicide.
- Cap, diaphragm, or sponge with spermicide.
- Double-barrier method (i.e., male condom with either cap, diaphragm, or sponge with spermicide).

Unacceptable Methods of Contraception

Unacceptable methods of contraception include:

- Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods).
- Withdrawal (i.e., coitus interruptus).
- Spermicides only.
- Lactational amenorrhea.
- Use of female and male condoms together.
- No method at all.
- Cap, diaphragm/sponge without spermicide and without male condom.

Appendix 2. Protocol Amendments

Protocol Amendment # 1

Date of Amendment: June 21, 2024

The following updates were made based on regulatory feedback:

Section	Summary of Change	Justification
Section 7.2. Exclusion Criteria	Excluded participants with any specific investigational product safety concern	SAHPRA recommendation for participant safety
Section 7.2. Exclusion Criteria	Provided clarifying language regarding exclusion of participants with significant neuropsychiatric disease	SAHPRA recommendation ensuring study population of healthy volunteers
Section 7.2. Exclusion Criteria	Modified volume of blood loss to be excluded	SAHPRA recommendation for participant safety
Section 7.2. Exclusion Criteria	Excluded employees or close relatives of the Sponsor, CRO, third-party vendors, or affiliates of the above-mentioned parties	SAHPRA recommendation to exclude bias
Section 7.2. Exclusion Criteria	Excluded participants with inadequate venous access	SAHPRA recommendation as inability to draw blood may interfere with compliance to the study protocol
Section 7.2. Exclusion Criteria	Excluded vulnerable participants	SAHPRA recommendation for participant safety

Protocol Amendment # 2

Date of Amendment: August 29, 2024

The following key updates were made based on additional statistical considerations:

Section	Summary of Change	Justification
Section 5. Study Objectives and Endpoints	Selected singular primary endpoint and modified secondary endpoints accordingly	Aligned with regulatory acceptance of prior art
Section 7.1. Inclusion Criteria	Modified weight of inclusion	Updated to mitigate risks of blood loss during trial
Section 8.6.1. Prior and Concomitant Medication	Added daily iron supplementation from discharge of treatment period 1 and continuing for 60 days	Provided to mitigate risks of blood loss during trial
Section 11.1.2. Pharmacodynamic Evaluation	Removed assessment of corrected C-Peptide measurements	Aligned with planned assessments
Section 12.5. Injection Site Assessment	Corrected assessment description	Aligned with planned eCRF
Section 12.6.1.3. Device Reportable Adverse Event	Added description of device reportable AE	Updated for completeness
Section 12.6.1.4. Pre-treatment Adverse Event	Removed definition of pre-treatment AE	Updated for consistency
Section 12.6.6. Insulin Pen Adverse Event Reporting	Added instructions for reporting insulin pen related AEs	Updated for completeness
Section 14. Statistical Analysis	Provided fixed sequence of endpoints	Controlled for multiplicity
Section 14.1. Determination of Sample Size	Modified sample size and provided justification	Aligned with regulatory acceptance of prior art
Section 14.2. Analysis Populations	Adjusted PK Concentration Population, PK Parameter Population, and PD Parameter Population	Selected participants without major protocol deviations
Section 14.3. Pharmacokinetic Analysis	Updated based on endpoint modification	Aligned with regulatory acceptance of prior art
Section 14.4 Pharmacodynamic Analysis	Updated based on endpoint modification and adjusted confidence interval	Aligned with regulatory acceptance of prior art