Clinical Development

LYSAKARE®

CAAA001A12401 / NCT04524442

A multicenter, open-label post authorization safety study to evaluate the effect of LysaKare® infusion on serum potassium levels in GEP-NET patients eligible for Lutathera® treatment.

Statistical Analysis Plan (SAP)

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			Remove signature page as approval will be done in Subway	Signature Page
			Updated Figure 1-1 Study design	1.1 Study Design
			Added reference to the subgroup with non-serum potassium levels	2.2.1 Subgroup of interest
			Added the definition of dose compliance.	2.4.1 Dose compliance
			Added reference to the Primary Analysis Evaluable Set and the separate analyses planned for the non-serum potassium samples	2.5.1 Primary endpoint(s)
			Added the definition of prior medication	2.4.2 Prior, concomitant and post therapies
			Added a plan of supplementary analysis utilizing CTC graded potassium values measured from all sources	2.5.6 Supplementary analyses
			Updated Table 2-1 Hematology and Chemistry Laboratory Assessments	2.7.3 Laboratory Data
			Added details of analyzing the subgroup with non-serum potassium values	2.12 Other Exploratory analyses
			Updated language to align with Protocol Amendment v1.0	3 Sample size calculation

Table of contents

List of tables

List of figures

List of abbreviations

AE	Adverse event
AESI	Adverse Events of Special Interest
ALP	Alkaline Phosphatase
ALT/ALAT	Alanine Aminotransferase
AST/ASAT	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Classification
BMI	Body Mass Index
CI	Confidence Interval
CSR	Clinical Study Report
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
GEP-NET	Gastro-Entero-Pancreatic Neuroendocrine Tumors
h	Hour
HCO3	Bicarbonate
IV	Intravenous
LDH	Lactate Dehydrogenase
MAR	Missing at Random
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
mL	Milliliter
NCI	National Cancer Institute
PASS	Post Authorization Safety Study
pCO2	Partial Pressure of Carbon Dioxide
PT	Preferred Term
RAP	Report and Analysis Process
SAE	Serious Adverse Event
SAF	Safety Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SSR	Somatostatin Receptor
TEAE	Treatment Emergent Adverse Event
TFLs	Tables, Figures, Listings
TNM	Tumors, Nodes, Metastases
ULN	Upper Limit of Normal
WBC	White Blood Cells
WHO	World Health Organization
WHO-DRL	World Health Organization-Drug Reference List

1 Introduction

The purpose of this Statistical Analysis Plan (SAP) is to outline the statistical methods and document the outputs needed for the clinical study report (CSR) of the primary analysis of study CAAA001A12401, a multicenter, open-label, post authorization safety study (PASS) to evaluate the effect of LysaKare® infusion on serum potassium levels in Gastroenteropancreatic neuroendocrine tumors (GEP-NET) patients eligible for Lutathera® treatment.

The content of this SAP is based on protocol CAAA001A12401 version 01 (release date: 01-Dec-2022). All decisions regarding primary analysis, as defined in the SAP document, have been made prior to database lock of the study data.

1.1 Study design

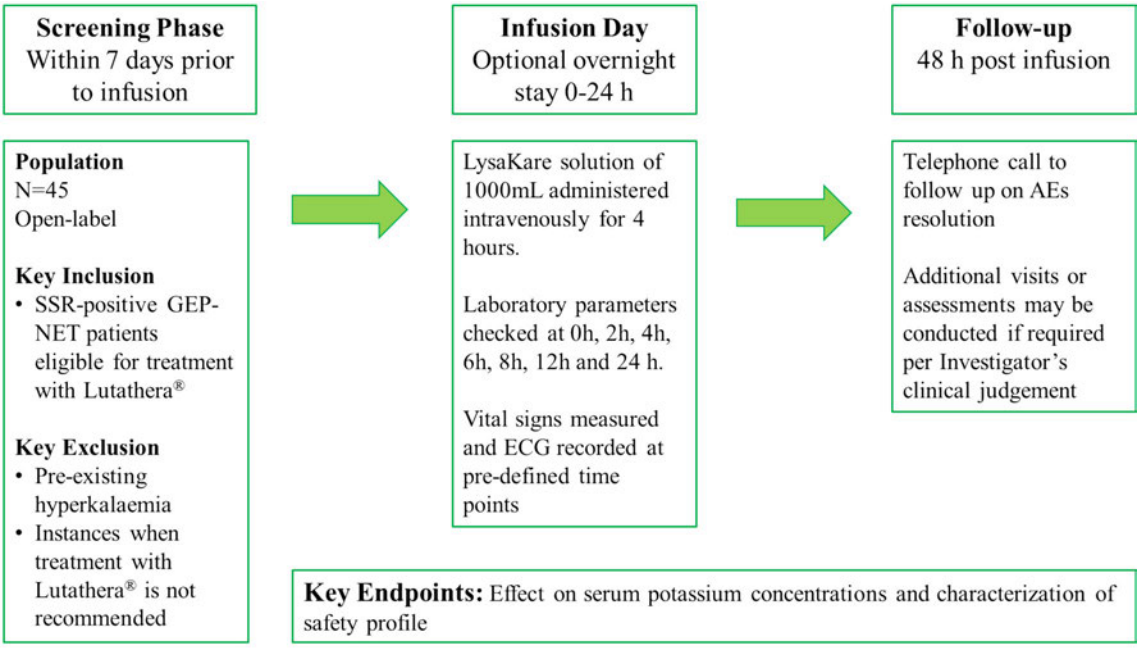
This is a multi-center, open-label PASS to evaluate the effect of LysaKare® infusion on serum potassium levels in GEP-NET patients eligible for Lutathera® treatment. Eligible patients will be admitted to the in-clinic unit to be dosed with LysaKare® solution for infusion of 1,000 mL, which is administered intravenously over a period of 4 hours. Before the infusion (at 0 h time point), a set of baseline tests will be performed. During and after the infusion, patient condition will be monitored for evaluation of any adverse events (AEs). The main purpose of the study is to assess the effect of LysaKare® administration on serum potassium concentration in GEP-NET patients eligible for Lutathera® treatment.

As per protocol version 01, approximately 45 patients (to have at least 25 patients evaluable for the primary endpoint) with GEP-NET will be enrolled to receive one infusion with LysaKare® to systematically assess the effect of LysaKare® administration on potassium blood level concentration up to 24 h compared to baseline.

The study schedule for each patient consists of a screening period followed by an infusion day with an optional overnight in-clinic stay, and a follow up call as described in Figure 1-1.

The primary analysis is planned to be performed after the last enrolled patient has completed the follow-up phone contact after LysaKare® infusion.

Figure 1-1 Study design



1.2 Study objectives, endpoints and estimands

Objectives and related endpoints are described in Table 1-1 below.

Table 1-1 Objectives and related endpoints

Objectives	Endpoints
Primary objective	**Endpoint for primary objective**
To assess the effect of LysaKare® administration on serum potassium concentration in GEP-NET patients eligible for Lutathera® treatment	Change in serum potassium levels at specified time points after LysaKare® Intravenous (IV) administration compared to baseline.
Secondary objective	**Endpoints for secondary objective(s)**
To characterize the safety profile on LysaKare® infusion in GEP-NET patients eligible for Lutathera® treatment without co-administration of Lutathera®	• Incidence of LysaKare® related AEs • Changes in vital signs and Electrocardiogram (ECG) parameters • Change in laboratory parameters

1.2.1 Primary estimand

The primary clinical question of interest is: What is the effect of LysaKare® administration on serum potassium levels in Gastroenteropancreatic neuroendocrine tumors (GEP-NET) patients eligible for Lutathera® treatment regardless of treatment discontinuation or interruption for any reason?

The justification for the primary estimand is that it will capture the effect on serum potassium concentration that would be seen when LysaKare® administration is given to reduce renal radiation exposure in GEP-NET patients eligible for Lutathera® treatment.

The primary estimand is described by the following attributes:

1. **The target population** comprises male and female patients who are 18 years or older diagnosed with SSR positive GEP-NET and eligible for treatment with Lutathera®, who received LysaKare®.
2. **The primary variable** is the change in serum potassium concentration from baseline to 2, 4, 6, 8, 12, and 24 hours after beginning of LysaKare® administration.
3. **The treatment of interest** is LysaKare® solution administered intravenously over a period of four hours.
4. **The intercurrent event** of interest is treatment interruption or discontinuation for any reason. This intercurrent event is ignored (treatment policy strategy).
5. **The summary measure** is the mean change with associated 95% Confidence interval (CI) in serum potassium levels from baseline to 2, 4, 6, 8, 12 and 24 hours after beginning of LysaKare® administration.

1.2.2 Secondary estimand(s)

Not applicable.

2 Statistical methods

2.1 Data analysis general information

All analyses will be performed by Novartis. SAS version 9.4 or later software will be used to perform all data analyses and to generate tables, figures and listings.

Data included in the analysis

The analysis cut-off date for the primary endpoint analysis is defined as the date when all enrolled patients will have completed the phone call to follow-up on AEs.

All statistical analyses will be performed using all data collected in the database up to the data cut-off date. All data with an assessment date or event start date (e.g., start date of an AE) prior to or on the cut-off date will be included in the analysis. Any data collected beyond the cut-off date will not be included in the analysis and will not be used for any derivations.

All events with start date before or on the cut-off date and end date after the cut-off date will be reported as ‘ongoing’. The same rule will be applied to events starting before or on the cut-off date and not having a documented end date. This approach applies, in particular, to AEs and concomitant medication reports. For these events, the end date will not be imputed and therefore will not appear in the listings.

General analysis conventions

Pooling of centers: Unless specified otherwise, data from all study centers will be pooled for the analysis. However, if any center enrolls a large number of patients, a subgroup analysis by centers will be considered. For the subgroup analysis, lower enrolling centers will be pooled.

Qualitative data (e.g., gender, race (where applicable), etc.) will be summarized by means of contingency tables; a missing category will be included as applicable. Percentages will be calculated using the number of patients in the relevant population as the denominator.

Quantitative data (e.g., age, body weight, etc.) will be summarized by appropriate descriptive statistics (i.e., mean, standard deviation, median, minimum, maximum, and quartiles [where appropriate]).

2.1.1 General definitions

Study treatment

The study treatment, used throughout the protocol and SAP, will refer to the solution of LysaKare® administered intravenously. Only one infusion will be given in the treatment phase of the trial.

Date of administration of study treatment

The date and time of administration of study treatment is derived as the start date and time of the infusion. The date and time of administration of study treatment will also be referred as ***start of study treatment***.

Study day

The study day describes the day of the event or assessment date, relative to the reference start date.

The study day is defined as:

- The date of the event (visit date, onset date of an event, assessment date, etc.) − reference start date + 1 if event is on or after the reference start date.
- The date of the event (visit date, onset date of an event, assessment date, etc.) − reference start date if event precedes the reference start date.

The reference start date for all assessments (e.g., AE onset, laboratory abnormality occurrence, vital sign measurement, etc.) is the start of study treatment.

Time units

A year length is defined as 365.25 days. A month length is 30.4375 days (365.25/12). If duration is reported in months, duration in days will be divided by 30.4375. If duration is reported in years, duration in days will be divided by 365.25.

Baseline

For safety evaluations, the last available assessment on or before the date of start of study treatment is defined as "baseline" assessment.

For cases where time of assessment is captured (e.g., pre-dose ECG, laboratory assessments), the last available assessment before the treatment start date/time is used for baseline.

In rare cases where multiple measurements meet the baseline definition, with no further flag or label that can identify the chronological order, then the following rule should be applied: For ECGs or vital signs, the last value will be considered as baseline.

For the assessment of change in electrolytes and blood gas parameters, the baseline is defined as the assessment 0 hr (i.e., Pre-dose).

If participants have no value as defined above, the baseline results will be considered missing.

On-treatment assessment/event and observation periods

For AE reporting the overall observation period will be divided into three mutually exclusive segments:

1. ***pre-treatment period***: from day of patient's sign informed consent to the day and time before administration of study treatment.
2. ***on-treatment period***: from date and time of administration of study treatment to 30 days after date of last administration of study treatment.
3. ***post-treatment period***: starting at day 31 after administration of study treatment.

If dates are incomplete in a way that clear assignment to pre-, on-, and post-treatment period cannot be made, then the respective data will be assigned to the on-treatment period. Refer to Section 5.1.2 for imputation rules concerning AE start and stop dates.

Safety summaries (tables, figures) include only data from the on-treatment period with the exception of baseline data which will also be summarized where appropriate (e.g., change from baseline summaries). In addition, a separate summary for death including on treatment and post treatment deaths will be provided. In particular, summary tables for AEs will summarize only on-treatment events, with a start date during the on-treatment period (***treatment-emergent*** AEs).

However, all safety data will be listed and those collected during the pre-treatment and post-treatment period will be flagged.

Windows for multiple assessments

For the definition of time windows in the treatment phase, refer to the study protocol Section 8.4.2 and Table 8-2 of the Protocol. During the Treatment Phase (infusion day): a time window of +/- 5% (equal to 3 min/h) is permitted for study assessments at 2 h, 4 h, 6 h, 8 h, 12 h. A time window of +/- 2 hours is accepted for the 24-hour assessments. Study assessments captured outside of the time window will still be used in any analyses and will be captured as a minor protocol deviation.

In order to summarize physical exam, vital sign, ECG, laboratory collected over time (including unscheduled visits), the assessments will be time slotted. The following general rule will be

applied in creating the assessment windows: If more than one assessment is done within the same time window, the assessment performed closest to the target date will be used. If two assessments within a time window are equidistant from the target date, then the earlier of the two assessments will be used.

If multiple assessments occur on the same date, then the worst-case result will be used. Data from all assessments (scheduled and unscheduled), including multiple assessments, will be listed.

The last contact date is defined as when the enrolled patient has completed the follow-up phone contact 48 hours after LysaKare® infusion. The cut-off date will not be used for last contact date, unless the patient was seen or contacted on that date. No data post cut-off date will be used. Completely imputed dates (e.g., the analysis cut-off date programmatically imputed to replace the missing end date of a dose administration record) will not be used to derive the last contact date.

2.2 Analysis sets

Enrolled set

The enrolled set consists of all patients who signed informed consents and participated in the screening phase of the study.

Safety set

The safety set (SAF) comprises all patients who received any administration of the LysaKare® infusion in the study.

The safety set will be used for all analyses in this study, whereas the primary endpoint analysis is further restricted to an evaluable subset based on the following specification.

Evaluable set

Evaluable set comprises all patients who received any volume of the LysaKare® infusion and provided at least 2 potassium levels measured from serum: pre-dose value and at least one post-dose value from the post-treatment window where the acute serum potassium changes are expected (between 4 and 8 hours).

2.2.1 Subgroup of interest

Potassium levels as well as key safety (all AEs, AEs with suspected relationship to study treatment and SAEs) analyses will be repeated in the following subgroups:

- Age: 18-64 years vs. ≥ 65 years
- Gender : Male vs. Female
- Race: Asian vs. Black or African American vs. White vs. Other (only subgroups with at least 5 patients)
- Center: depending on whether there is any high recruiting center (only centers with at least 10 patients).

- Creatinine Clearance: < 60ml/min vs. ≥60- <90 ml/min vs. ≥ 90 ml/min (only subgroups with at least 5 patients).

The objective for carrying out these subgroup analyses is to identify potential safety issues that may be limited to a subgroup of patients, or safety issues that are more commonly observed in a subgroup of patients.

2.3 Patient disposition, demographics and other baseline characteristics

The SAF will be used for all baseline and demographic summaries and listings unless otherwise specified. No inferential statistics will be provided.

2.3.1 Patient disposition

Enrollment by country and center will be summarized for all screened patients. The number and percentage of patients included in the SAF will be presented. The number (%) of screened and not-treated patients and the reasons for screening failure will also be displayed. The number (%) of patients in the SAF who completed the study follow-up period, who discontinued the study follow-up and the reason for discontinuation will be presented.

Protocol deviations

The number (%) of patients in the SAF with any major protocol deviation will be tabulated by deviation category (as specified in the study Data Management Plan). All protocol deviations will be listed.

Analysis sets

The number (%) of patients in each analysis set (defined in Section 2.2) will be summarized.

2.3.2 Demographics and other baseline characteristics

Basic demographic and background data

All demographic and baseline disease characteristics data will be summarized and listed. Categorical data (e.g., gender, age groups: <65 and ≥ 65 years, race, ethnicity) will be summarized by frequency counts and percentages; the number and percentage of patients with missing data will be provided. Continuous data (e.g., age, weight, height, body mass index [BMI]) will be summarized by descriptive statistics (N, mean, median, standard deviation, 25th and 75th percentiles, minimum and maximum). BMI (kg/m2) will be calculated as weight[kg] / (height[m2]) using weight at Baseline.

Diagnosis and extent of cancer

Summary statistics will be tabulated for diagnosis and extent of cancer. This analysis will include the following: time since initial GEP-NET tumor diagnosis, primary site of cancer, tumor, node, metastasis (TNM) criteria at screening date, disease stage, time since most recent

relapse/progression, GEP-NET grade according to Ki-67 index, presence of metastases at time of screening, and number and type of metastatic sites involved.

Medical history

Medical history and ongoing conditions will be summarized. Separate summaries will be presented for ongoing and historical medical conditions. The summaries will be presented by primary system organ class (SOC), and preferred term (PT). Medical history and current medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology. The MedDRA version used for reporting will be specified in the CSR and as a footnote in the applicable tables/listings.

Other

All data collected at baseline will be listed as appropriate.

2.4 Treatments (study treatment, rescue medication, concomitant therapies, compliance)

2.4.1 Exposure to study treatment

Duration of exposure to study treatment will be derived as follows:

Duration of exposure to study treatment (*hours*) = End time of LysaKare® infusion – Start time of LysaKare® infusion.

Duration of exposure, total duration of dose interruption as well as the actual total volume of the LysaKare® infusion, will be summarized. Duration of exposure will be categorized into time intervals (<1 hour. 1- <2 hours, 2- <3 hours and 40 minutes, 3 hours 40 minutes- 4 hours and 20 minutes, and > 4 hours and 20 minutes); frequency counts and percentages will be presented for the number (%) of subjects in each interval. Actual total volume will be categorized into volume intervals (< 250 mL, 250-499 mL, 500-749 mL, 750-899 mL, 900-1000 mL, > 1000 mL); frequency counts and percentages will be presented for the number (%) of subjects in each interval. The number (%) of subjects who have dose discontinuations prior to receiving at least 900 mL of LysaKare® will be summarized as well as the number of subjects with dose interruptions, with the associated reasons. The number (%) who received an anti-emetic before or during LysaKare® infusion will be presented.

The safety set will be used for all summaries and listings of study treatment.

2.4.2 Prior, concomitant and post therapies

Prior and concomitant anti-cancer therapy

The number and percentage of patients who received any prior anti-neoplastic medications, prior anti-neoplastic radiotherapy or underwent prior anti-neoplastic surgery will be summarized. Prior anti-neoplastic medications will be summarized by lowest ATC class, and PT.

Similarly, the number and percentage of patients who received any concomitant anti-neoplastic medications, concomitant anti-neoplastic radiotherapy or underwent any concomitant anti-neoplastic surgery will be summarized. Concomitant anti-neoplastic medications will be summarized by lowest ATC class, and PT.

Separate listings will be produced for prior and concomitant anti-neoplastic medications, radiotherapy, and surgery.

Anti-neoplastic medications will be coded using the World Health Organization (WHO) Drug Reference List (WHO-DRL). Details regarding WHO-DRL version will be included in the footnote in the tables/listings.

Concomitant therapy

Concomitant therapy is defined as all interventions (therapeutic treatments and procedures) other than the study treatment administered to a patient coinciding with the study treatment period. Concomitant therapy includes medications (other than study treatment) starting on or after the start date of study treatment or medications starting prior to the start date of study treatment and continuing after the start date of study treatment. Prior therapy includes medication of which the start date and end date both prior to the start date of study treatment.

Concomitant medications will be coded using the WHO-DRL dictionary that employs the WHO Anatomical Therapeutic Chemical (ATC) classification system and summarized by lowest ATC class and PT using frequency counts and percentages.

Surgical and medical procedures will be coded using MedDRA and summarized by SOC and PT.

All concomitant therapies will be listed. Any concomitant therapies starting and ending prior to the start of study treatment will be flagged in the listing. The safety set will be used for all concomitant medication tables and listings.

2.5 Analysis supporting primary objective(s)

The primary objective of the study is to evaluate the effect of LysaKare® administration on serum potassium concentration in GEP-NET patients eligible for Lutathera® treatment.

2.5.1 Primary endpoint(s)

The primary endpoint of the study is change in the serum potassium levels at specified time points after LysaKare® IV administration compared to baseline. The primary analysis will be performed on the Evaluable Set after the last enrolled patient has completed the follow-up phone contact after LysaKare® infusion.

Potassium testing will be performed on the infusion day at the following timepoints: 0 h (before the start of the infusion), 2 h, 4 h, 6 h, 8 h, 12 h, and 24 h (after the start of the infusion).

2.5.2 Statistical hypothesis, model, and method of analysis

The study is descriptive in nature, and no hypothesis will be tested for study endpoints. The study will analyze and describe various parameters related to serum potassium concentration,

including mean change, maximum change, and time to the maximum change. In particular, maximum change and time to the maximum change will be summarized by descriptive statistics (N, median, minimum, maximum, and interquartile range).

The mean (+/- SD) concentration-time profiles for serum potassium concentration by treatment over time will be displayed graphically for the Evaluable set on the linear view.

To visualize the evolution of potassium level for each patient a spaghetti plot will be displayed along with a box plot to see the summary of potassium levels at each time point.

2.5.3 Handling of intercurrent events

The two intercurrent events of interest, treatment discontinuation, and treatment interruption, will be handled the same, as the statistical analysis of the primary estimand will include all patients, regardless of whether a patient had a treatment discontinuation or interruption. However, if 3 or more (approximately 10%) patients from the Evaluable Set have experienced the considered intercurrent event, a supplementary analysis for the primary estimand will be conducted taking into account the following.

1. Statistical analysis of the primary estimand after removal of subjects with treatment discontinuation for any reason
2. Statistical analysis of the primary estimand after removal of subjects with treatment interruption causing the total infusion time > 4 h and 20 minutes for any reason.
3. Statistical analysis of the primary estimand after removal of subjects with either treatment discontinuation or interruption for any reason.

2.5.4 Handling of missing values not related to intercurrent event

There will be no replacement of missing data not related to intercurrent events.

2.5.5 Sensitivity analyses

The time course of the relative percent change from pre-dose to each time point will be tabulated. A Mixed Model Repeated Measures (MMRM) will be fitted to assess the whole-time course.

As a sensitivity analysis, a MMRM will be fitted with the relative percent change from pre-dose potassium value as dependent variable, and pre-dose potassium value, actual treatment volume, duration of infusion, timepoint, and actual treatment volume by timepoint interaction as covariates. This model implicitly imputes missing values by means of the estimated parameters and provides unbiased estimates for the data which are missing at random (MAR). Least-Squares Means and their 95% confidence intervals will be presented by timepoint and overall. A general (unstructured) variance-covariance matrix for the repeated measures for a single patient will be used.

This analysis will be repeated for absolute change from pre-dose.

Absolute change and relative percent change from pre-dose will be plotted against time to visualize the trend (increasing or decreasing) over time.

2.5.6 Supplementary analyses

Potassium level measured on whole blood does not account for potential hemolysis and thus may overestimate the actual level of potassium that would have been measured in serum. A supplementary descriptive analysis, similar to the primary analysis, including all post-infusion potassium levels, irrespective of the sample source, will be conducted to assess whether the increase in potassium is still acceptable despite the expected overestimation. Patients with pre-treatment potassium level measured in whole blood will be excluded from this analysis.

For the potassium levels measured at pre-infusion and any post-infusion time points, regardless of sample sources, categories based on normal range and Common Toxicity Criteria (CTC) grade will be summarized across the Safety Set at each time point to assess the frequency of elevated potassium levels.

To further assess the effect of LysaKare® infusion over a 24-hour period, the change in the other electrolyte and blood gas components post-infusion (Sodium, Chloride, pH, Lactate, CO_2, and HCO_3) will be analyzed. The primary analysis will be repeated for these parameters.

The primary analysis will be repeated for subgroups of interest described in Section 2.2.1.

2.6 Analysis supporting secondary objectives

The secondary objective is to confirm the safety profile of LysaKare® infusion in GEP-NET patients without co-administration of Lutathera®. Further discussion of the safety endpoints to support the secondary objective analysis is seen in Section 2.7.

No efficacy endpoints will be assessed in this study.

2.7 Safety analyses

All safety analyses will be based on the safety set.

2.7.1 Adverse events (AEs)

All AEs, whether or not spontaneously reported by the patient, will be recorded starting from the signing of the ICF until the end of the Follow-Up phase. Any SAEs experienced after the 2-day study period should only be reported if the investigator suspects a causal relationship to study treatment.

AEs are coded using MedDRA terminology. The latest MedDRA version used for reporting will be specified in the CSR and as a footnote in the applicable tables/listings. AEs will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 or current version.

AE summaries will include all AEs occurring during on-treatment period. All AEs collected in the AE (e)CRF page will be listed along with the information collected on those AEs e.g. AE relationship to study treatment, AE outcome etc. AEs with start date outside of on-treatment period will be flagged in the listings.

AEs will be summarized by number and percentage of subjects having at least one AE, having at least one AE in each primary system organ class and for each PT using MedDRA coding. A

patient with multiple occurrences of an AE will be counted only once in the respective AE category. A subject with multiple CTCAE grades for the same PT will be summarized under the maximum CTCAE grade recorded for the event. AE with missing CTCAE grade will be included in the 'All grades' column of the summary tables.

In AE summaries, the primary SOC will be presented in order of descending frequency and the PT will be sorted within primary SOC in descending frequency.

The following AE summaries will be produced:

- Overview of AEs and deaths (number and % of subjects who died, with any AE, any SAE, any dose interruptions, etc)
- AEs by SOC and PT, summarized by relationship (all AEs and AEs related to study treatment), seriousness (SAEs and non-SAEs), leading to treatment discontinuation, leading to dose interruption, requiring additional therapy and leading to death.
- In addition, a summary of AEs with number of occurrences will be produced (an occurrence is defined as >1 day between start and prior end date of record of same PT).

For the legal requirements of ClinicalTrials.gov and EudraCT, two required tables, on AEs which are not SAEs with an incidence greater than 5% and on SAEs and SAEs suspected to be related to study treatment, will be provided by SOC and PT on the safety set population.

If for a same subject, several consecutive AEs (irrespective of study treatment causality, seriousness and severity) occurred with the same SOC and PT:

- a single occurrence will be counted if there is ≤ 1 day gap between the end date of the preceding AE and the start date of the consecutive AE.
- more than one occurrence will be counted if there is > 1 day gap between the end date of the preceding AE and the start date of the consecutive AE.

For occurrence, the presence of at least one SAE / SAE suspected to be related to study treatment / non-SAE has to be checked in a block e.g., among AEs in a ≤ 1 day gap block, if at least one SAE is occurring, then one occurrence is calculated for that SAE.

The number of deaths resulting from SAEs suspected to be related to study treatment and SAEs irrespective of study treatment relationship will be provided by SOC and PT.

AEs, AEs with suspected relationship to study treatment and SAEs will also be analysed for subgroups of interest described in Section 2.2.1.

2.7.1.1 Adverse events of special interest (AESI)

Hyperkalemia is the only AESI. The search strategy will be MedDRA PTs Hyperkalaemia and Blood potassium increased.

2.7.2 Deaths

Separate summaries for on-treatment and all deaths (including post-treatment death) will be produced by SOC and PT.

All deaths will be listed, post treatment deaths will be flagged. A separate listing of deaths prior to starting treatment will be provided for all screened subjects.

In case a patient died due to COVID-19, it must be clear from the eCRF if the death was due to underlying COVID-19 or a different reason. Therefore, 'Primary Cause/ Reason for Death' should indicate 'Suspected COVID-19' or 'Confirmed COVID-19'. If a test is performed after the patient died and the assessment indicates death was due to COVID-19 infection, the 'Primary Cause/ Reason for Death' should be retrospectively changed from 'Suspected COVID-19' to 'Confirmed COVID-19'. A separate presentation of deaths due to COVID-19 will be produced.

2.7.3 Laboratory data

On analyzing laboratory data, data from all sources will be combined. The summaries will include all assessments available for the lab parameters collected no later than 24 hours after the start of the LysaKare® infusion. The laboratory parameters that will be investigated, and the timing that the laboratory parameters will be assessed are summarized in Table 2-1.

Table 2-1 Hematology and Chemistry Laboratory Assessments

Haematology (Assessment at screening, pre-dose and 24h after start of the infusion)	**Blood Chemistry (serum)** (Assessment at screening, pre-dose and 24h after start of the infusion)	**Electrolytes (serum)** (Assessment at screening, pre-dose and 2h, 4h, 6h, 8h, 12h and 24h after start of the infusion)	**Venous Blood Gas** (Assessment at screening, pre-dose and 2h, 4h, 6h, 8h, 12h and 24h after start of the infusion)
WBC with differential	BUN or urea	Potassium	pH
Platelets	Uric acid	Sodium	Lactate
Haemoglobin	Serum creatinine	Chloride	pCO2
Haematocrit	Creatinine clearance*	HCO3	
	Albumin		
	Total bilirubin		
	Alkaline phosphatases		
	AST/ASAT		
	ALT/ALAT		
	Gamma-GT		
	Glucose		
	LDH		

(*) Calculated using Cockcroft-Gault formula

Laboratory values outside normal ranges will be listed and flagged. The following flags will be used:

'+': 'Higher than reference value'

'-': 'Lower than reference value'

The respective parameter and the flagged value will be presented patient-wise by visit together with the reference values.

The following summaries will be produced for hematology and biochemistry laboratory data (by laboratory parameter and treatment):

- Worst post-baseline Common Toxicity Criteria (CTC) grade (regardless of the baseline status). Each subject will be counted only for the worst grade observed post-baseline.
- Shift tables using CTC grades to compare baseline to the worst on-treatment value.
- For laboratory tests where CTC grades are not defined, shift tables using the low/normal/high/(low and high) classification to compare baseline to the worst on-treatment value.

The following listings will be produced for the laboratory data:

- Listings of all laboratory data, with CTC grades and classification relative to the laboratory normal range. Lab data collected during the post-treatment period will be flagged.
- Listing of all CTC grade 3 or 4 laboratory toxicities.

Liver parameters

Liver parameters of interest are total bilirubin (TBL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The number (%) of patients with worst post-baseline values as per Novartis Liver Toxicity guidelines will be summarized:

The following summaries will be produced:

- ALT or AST > 3xULN
- ALT or AST > 5xULN
- ALT or AST > 20xULN
- TBL > 2xULN
- TBL > 3xULN
- ALT or AST > 3xULN & TBL > 2xULN
- ALT or AST > 3xULN & TBL > 2xULN & ALP < 2xULN (potential Hy's law)

Potential Hy's Law events are defined as those patients with occurrence of AST or ALT > 3xULN and TBL > 2xULN, and ALP < 2xULN at initial presentation during the on-treatment period. Note that the criteria relating to combined elevations of AST (or ALT) and TBL are based on the peak values at any post-baseline time for a patient.

For patients with abnormal ALT or AST baseline values, a clinically significant liver safety signal corresponding to Hy's law is defined by: [ALT or AST > 3*baseline] OR [ALT or AST >8*ULN], whichever is lower, combined with [TBIL >2*baseline AND >2*ULN].

2.7.4 Other safety data

2.7.4.1 ECG data

Single standard 12-lead ECGs will be obtained using an ECG machine that automatically calculates heart rate (HR) and measures RR, PR, QRS, QT, and QTcF intervals. All ECG

assessments will be performed in the supine position. All ECG assessments will be read and interpreted locally. ECGs will be reported at screening, and at the 0h (before LysaKare® injection) as well as the 4h, 8h, and 24h timepoints (after LysaKare® injection).

Data handling

In case of ECG replicates at any assessment, the average of the ECG parameters at that assessment should be used in the analyses.

Data analysis

The number and percentage of subjects with notable ECG values will be presented.

- QT, QTcF
  - New value of > 450 and ≤ 480 ms
  - New value of > 480 and ≤ 500 ms
  - New value of > 500 ms
  - Increase from Baseline of > 30 ms to ≤ 60 ms
  - Increase from Baseline of > 60 ms
- HR
  - Increase from baseline >25% and to a value > 100 bpm
  - Decrease from baseline >25% and to a value < 50 bpm
- PR
  - Increase from baseline >25% and to a value > 200 ms
  - New value of > 200 ms
- QRS
  - Increase from baseline >25% and to a value > 120 ms
  - New values of QRS > 120 ms

The summaries will include all ECG assessments performed no later than 30 days after the last study treatment date.

A listing of all ECG assessments will be produced and notable (e.g. clinically significant abnormality) values will be flagged. In the listing, the assessments collected during the post-treatment period will be flagged.

For each of the ECG parameters, descriptive statistics at baseline, at each post-baseline time point and changes from baseline at each post-baseline time point will be summarized. Changes from baseline to maximum for QTcF will also be summarized.

Patients with notable ECG interval values will be listed and the corresponding notable values and abnormality findings will be included in the listings.

Unscheduled ECG measurements will not be used in computing the descriptive statistics for change from baseline at each post-baseline time point. However, they will be used in the analysis of notable ECG values.

2.7.4.2 Vital signs

Physical examinations will be performed by the Investigator, or qualified designee. All body systems will be examined, and any relevant findings will be documented in the source documents and eCRF. Physical examinations should include weight measurement (height will only be measured at baseline). Vital signs measurements should include heart rate, blood pressure and respiratory rate, and will be performed after the patient rests for 5 minutes. For each patient, all blood pressure recordings shall be made using the same type of instrument (i.e., manual BP recording vs. automatic digital vital signs monitor) on the same arm.

Data handling

Vital signs collected on treatment will be summarized. Values measured outside of on treatment period will be flagged in the listings.

Data analysis

For analysis of vital signs, the clinically notable vital sign criteria are provided in Table 2-2 below.

Table 2-2 Clinically notable changes in vital signs

Vital sign (unit)	Clinically notable criteria	
	Above normal value	**Below normal value**
Weight (kg)	increase > =10% from Baseline	decrease > 10% from Baseline
Systolic blood pressure (mmHg)	>=180 with increase from baseline of >=20	<=90 with decrease from baseline of >=20
Diastolic blood pressure (mmHg)	>=105 with increase from baseline of >=15	<=50 with decrease from baseline of >=15
Pulse rate (bpm)	>=100 with increase from baseline of >25%	<=50 with decrease from baseline of > 25%

The number and percentage of subjects with notable vital sign values (high/low) will be presented. Descriptive statistics will be tabulated for baseline, at each post-baseline time point and changes from baseline at each post-baseline time point for each vital sign measure.

A listing of all vital sign assessments will be produced, and notable values will be flagged. In the listing, the assessments collected outside of on-treatment period will be flagged.

2.8 Pharmacokinetic endpoints

Not applicable.

2.9 PD and PK/PD analyses

Not applicable.

2.10 Patient-reported outcomes

Not applicable.

2.11 Biomarkers

Not applicable.

2.12 Other Exploratory analyses

Not applicable.

2.13 Interim analysis

Not applicable.

3 Sample size calculation

The primary endpoint is the change in serum potassium levels after LysaKare® IV administration compared to baseline. In a previous study, which assessed hourly potassium changes in 11 patients treated with LysaKare®, peak concentration was observed at 2-4 h post-baseline, the mean baseline level was 4.2 mmol/L, mean increase at 2 h was 0.6 mmol/L and at 4 h it was 0.9 mmol/L (SD=0.3); the maximum increase was 1.5 mmol/L (Krenning et al., 2000). The mild increase in potassium levels did not manifest in any safety issues or clinically significant events. Since the maximum mean increase was observed at 4h, sample size calculations were based on the expected mean change in serum potassium levels at the 4h timepoint.

The current study will describe the expected mean change at 4h by a point estimate and the corresponding 95% confidence interval. In historical data a mean increase from baseline to 4h of 0.9 mmol/L was observed with a standard deviation of 0.3.

The criterion for choice of sample size will be based on length of confidence interval of the mean change at 4h calculated using the t-distribution. A requirement was initially chosen that the confidence interval has a total length not exceeding 0.2 mmol/L assuming the historical standard deviation to be valid, i.e. the distance should be no more than 0.1 mmol/L from the mean to the limit of the confidence interval when the estimated standard deviation is 0.3.

The initially estimated sample size of 38 subjects produces a two-sided 95% confidence interval with a distance from the mean to the limits that is equal to 0.099 when the estimated standard deviation is 0.3. With the required minimum number of 25 evaluable subjects for the primary endpoint analysis, the confidence interval total length is maintained with a slight increase to 0.25 mmol/L.

Additionally, the originally estimated sample size of 38 implies an adequate probability of observing common and very common AEs, see Table 3-1 below. This estimate holds true because the current laboratory sampling issue only impacts the evaluable number for the primary endpoint. Other safety assessments based on AEs remain evaluable for the whole safety set.

Table 3-1 Likelihood of observing at least one case for given sample size and incidence.

Sample size	Incidence of Adverse Event	
	5%	10%
10	0.401	0.651
20	0.642	0.878
30	0.785	0.958
38	0.858	0.982
40	0.871	0.985

The table displays the probability of observing at least one case of a certain AE which has the incidence of 5% or 10% in this patient population for the sample sizes of 10, 20, 30, 38, and 40. Any further increase in total sample size ensures that this probability remains above 87%.

Since the sample size of 38 assumes no drop-out, additional subjects have been added to hedge against drop-out. Given the design of the study, no or very minor drop-out is expected. The additional precaution is taken to ensure at least 25 subjects are evaluable for the primary endpoint analysis as discussed in the amendment rationale. Therefore, the adjusted sample size is approximately 45 subjects.

4 Change to protocol specified analyses.

4.1 Impact of COVID-19

Due to pandemic COVID in year 2020, some extra analyses might be performed outside protocol specified analyses, if applicable.

Operational impact: Specified protocol deviation categories will be assigned to important deviations related to COVID-19 (e.g., treatment interruptions) and these will be summarized and listed separately. A summary table of the COVID-19 related deviations by relationship category will be provided.

Safety: To help the evaluation of the impact of the pandemic on safety, the incidence of COVID-19 related AE PTs will be presented incorporating any and all COVID-19 related AEs occurring before data cut-off. A listing of all COVID-related AEs will also be provided.

5 Appendix

5.1 Imputation rules

5.1.1 Study treatment

Patients with missing start dates are to be considered missing for all study treatment component related calculations and no imputation will be made. If start date is missing, then end-date should not be imputed.

5.1.2 AE and Concomitant medication (CM) date imputation

Any AEs and CMs with partial/missing dates will be displayed as such in the data listings.

Any AEs and CMs which are continuing as per data cut-off will be shown as 'ongoing' rather than the end date provided.

The imputations summarized in Table 5-1 and Table 5-2 are only used for analyses of time to and duration of AEs and concomitant medications

Table 5-1 Imputation of start dates (AE, CM) and assessments (LB, EG, VS)

Missing Element	Rule
day, month, and year	• No imputation will be done for completely missing dates

day, month	• If available year = year of study treatment start date, then
	  • If stop date contains a full date and stop date is earlier than study treatment start date, then set start date = 01JanYYYY
	  • Else set start date = study treatment start date.
	• If available year > year of study treatment start date, then 01JanYYYY
	• If available year < year of study treatment start date, then 01JulYYYY

Day	• If available month and year = month and year of study treatment start date, then
	  • If stop date contains a full date and stop date is earlier than study treatment start date, then set start date= 01MONYYYY.
	  • Else set start date = study treatment start date.
	• If available month and year > month and year of study treatment start date, then 01MONYYYY
	• If available month and year < month year of study treatment start date, then 15MONYYYY

Table 5-2 Imputation of end dates (AE, CM)

Missing Element	Rule (*=last treatment date plus 30 days not > (death date, cut-off date, withdrawal of consent date))
day, month, and year	• Completely missing end dates (incl. ongoing events) will be imputed by the end date of the on-treatment period* • If start date is after last treatment date plus 30 days then impute to the earliest date between death date, cut-off date and withdrawal of consent date.
day, month	• If partial end date contains year only, set end date = earliest of 31DecYYYY or end date of the on-treatment period *
Day	• If partial end date contains month and year, set end date = earliest of last day of the month or end date of the on-treatment period *

5.1.2.1 Prior therapies date imputation

Any prior anti-cancer therapies with partial/missing dates will be displayed as such in the data listings.

5.1.2.2 Post therapies date imputation

Not applicable.

5.1.2.3 Other imputations

Incomplete date of initial diagnosis of cancer and date of most recent recurrence

Missing day is defaulted to the 15th of the month and missing month and day is defaulted to 01-Jan.

5.2 AEs coding/grading

AEs are coded using the MedDRA terminology. The latest available MedDRA version at the time of the analyses should be used.

AEs will be assessed according to the CTCAE version 5.0 or more recent version.

The CTCAE represents a comprehensive grading system for reporting the acute and late effects of cancer treatments. The CTCAE grading is, by definition, a 5-point scale generally corresponding to mild, moderate, severe, life threatening, and death. This grading system inherently places a value on the importance of an event, although there is not necessarily proportionality among grades (a grade 2 is not necessarily twice as bad as a grade 1).

5.3 Laboratory parameters derivations

Grade categorization of lab values will be assigned programmatically as per National Cancer Institute (NCI) CTCAE version 5.0 or more recent version. The calculation of CTCAE grades will be based on the observed laboratory values only, clinical assessments will not be taken into account. The criteria to assign CTCAE grades are given in Novartis internal criteria for CTCAE grading of laboratory parameters. The latest available version of the document based on the underlying CTCAE version 5.0 or more recent version at the time of analysis will be used (refer to Table 5-3 in the Novartis internal criteria for CTC grading of laboratory parameters). For laboratory tests where grades are not defined by CTCAE v5.0 or more recent version, results will be graded by the low/normal/high classifications based on laboratory normal ranges.

A severity grade of 0 will be assigned for all non-missing lab values not graded as 1 or higher. Grade 5 will not be used. For laboratory tests that are graded for both low and high values, summaries will be done separately and labelled by direction, e.g., sodium will be summarized as hyponatremia and hypernatremia.

Imputation Rules

CTC grading for blood differentials is based on absolute values. However, this data may not be reported as absolute counts but rather as percentage of WBC.

If laboratory values are provided as ‘<X’ (i.e. below limit of detection) or ‘>X’, prior to conversion of laboratory values to SI unit, these numeric values are set to X.

The following rules will be applied to derive the WBC differential counts when only percentages are available for a xxx differential,

xxx count = (WBC count) * (xxx %value / 100)

Further derivation of laboratory parameters might be required for CTCAE grading. For instance, corrected calcium can be derived using the reported total calcium value and albumin at the same assessment using the following formula:

Corrected Calcium (mg/dL) = Calcium (mg/dL) – 0.8 [Albumin (g/dL)-4]

In order to apply the above formula, albumin values in g/L will be converted to g/dL by multiplying by 0.1), calcium values in mmol/L will be converted to mg/dL by dividing by 0.2495. For calculation of laboratory CTC grades 0 and 1, the normal range for derived corrected calcium is set to the same limits (in mg/dL) as for calcium.

CTC grades for the derived absolute WBC differential counts (neutrophils, lymphocytes) and corrected calcium will be assigned as described above for grading.

5.4 Statistical models

5.4.1 Analysis supporting primary objective(s)

Longitudinal data using mixed models will be preferably fitted on change from baseline (relative changes defined as the percent change of post-baseline value over baseline value, log2 transformation may be applied to the relative changes if distribution is right-skewed) with baseline (also log2 transform if distribution is right skewed), time (e.g. theoretical time since dosing) and the actual treatment volume by time interaction as fixed effect.

To account for correlated repeated measures within patients an unstructured variance-covariance matrix ('type=UN') will be used unless there is adequate justification in using an alternative variance-covariance matrix such as a power spatial covariance structure (type= 'sp(POW)') for unequally spaced data or a random subject effect.

Note: Although the unstructured covariance structure is the most robust variance-covariance matrix, in many cases, the number of parameters within the unstructured variance-covariance structure may cause your model to be unstable and thus a choice, such as the spatial covariance options, may provide a better fit to your data without having to fit so many parameters.

In SAS PROC MIXED, the repeated time effect should be explicitly included in the repeated statement. If this optional variable is not included, SAS assumes that the repeated measures data is similarly ordered for each subject. In addition, all missing response variables are indicated with periods in the input data set unless they all fall at the end of a subject's repeated response profile. Thus, as good standard practice we will include this repeated time effect explicitly. i.e **REPEATED <repeated-effect></options>;**

Relevant adjusted means and associated confidence intervals will be derived.

Standard model checking (e.g. residuals, random effects) will be performed to ensure that the basic assumptions of the model are not violated.

Alternative model making assumptions about the nature of the effects (e.g. linear effect of dose) may be investigated to better describe the changes observed.

5.4.2 Analysis supporting secondary objective(s)

Hyperkalemia and dose interruption/withdrawal events will be summarized in terms of percentage rates with 95% CIs. An exact binomial confidence interval (implemented using SAS procedure FREQ with EXACT statement for one-way tables) will be calculated (Clopper & Pearson, 1934)

SAS procedure FREQ will be used to estimate the proportion of subjects with hyperkalemia and dose interruption/withdrawal events (binary outcome = 1 or "Yes"), along with the associated 95% (=100 × (1 – *two-sided alpha level*)) two-sided Pearson-Clopper CI.

6 References

Clopper, C. J., & Pearson, E. S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrical, 26, 404-413

Krenning, E. P., De Jong, M., & Valkema, R. (2000). Inhibition of renal uptake of radiomolecules with a combinaiton of lysine and arginine. European Patent Specification (EP 1 196 154 B1).