Statistical Analysis Plan

Sponsor:	VectivBio AG
Protocol No:	TA799-015
Protocol Title:	A Phase 1, Open-Label Evaluation of the Pharmacokinetics and Safety of a Single Dose of Apraglutide in Subjects with Normal and Impaired Hepatic Function
PRA Project ID:	VVB22467-22467X
Version Date:	03-Apr-2023 (SAP)

1.0 Approvals

The undersigned have approved this Statistical Analysis Plan for use in this study.

Name of Sponsor Representative / Title:	[redacted] / Director, Program Statistician
Signature of Sponsor Representative / Date:	[redacted] REASON: I approve this document 34644e60-2833-4f37-a03b-141ee3871433
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2.0 Table of Contents

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3.0 Introduction

This Statistical Analysis Plan (SAP) describes the statistical methods that will be used during the analysis and reporting of data collected under VectivBio Protocol TA799-015.

This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan has been developed using the protocol dated 06-Oct-2022 and the final eCRF(s) dated.

An approved and signed SAP is a requirement for database lock.

This SAP only covers the results that will be processed by the ICON Early Clinical & Bioanalytical Services (IEB) Biostatistics Department and Quantitative Pharmacology & Pharmacometrics (department calculating the pharmacokinetic (PK) parameters).

ICON IEB will perform the pharmacokinetic (PK), and safety and tolerability evaluation.

This SAP supersedes the statistical considerations identified in the protocol; where considerations are substantially different, they will be so identified. Any post-hoc or unplanned analyses, or significant changes from the planned analysis in this SAP performed to provide results for inclusion in the clinical study report (CSR) but not included in this SAP, will be clearly identified in the CSR. Changes to planned analyses do not require an updated SAP but should be included in the CSR if significant.

4.0 Changes from Previous Version of Approved SAP

This is the first version of the SAP.

5.0 Study Objectives and Endpoints

5.1 Primary Objectives

- Part 1: To assess the Pharmacokinetic (PK) of apraglutide in subjects with moderate hepatic impairment compared with matched control subjects with normal hepatic function following single Subcutaneous (SC) dose administration.
- Part 2 (if applicable; see criteria to move to Part 2): To assess the PK of apraglutide in subjects with mild hepatic impairment compared with matched control subjects following single SC dose administration.

5.1.1 Primary Endpoint

- Area under the curve to infinity (AUC_{inf}) or area under the curve from time zero to the last quantifiable concentration (AUC_{last}) if AUC_{inf} cannot be reliably estimated.
- Area under the curve from time zero to 168 hours after apraglutide administration ($AUC_{0\text{-}168hr}$)
- Maximum observed plasma concentration (C_{max})

5.2 Secondary Objective

- To assess the safety and tolerability of apraglutide administered to subjects with impaired and normal hepatic function

5.2.1 Secondary Endpoint

- Time of maximum plasma concentration (t_{max})
- Apparent clearance after extravascular administration (CL/F)
- Apparent volume of distribution after extravascular administration (V_z/F)

- Terminal elimination rate constant (λ_z)
- Terminal half-life ($t_{1/2}$)
- Physical examination
- The incidence, nature, and severity of adverse events (AE) with apraglutide
- Changes in clinical laboratory results (chemistry, hematology, coagulation, and urinalysis) during and following trial treatment administration
- Changes in vital signs and 12-lead electrocardiogram (ECG) during and following trial treatment administration

6.0 Study Design

This is a Phase 1, non-randomized, open-label, single-dose trial to evaluate the effect of impaired hepatic function on the PK, safety, and tolerability of apraglutide administered to trial subjects via SC injection.

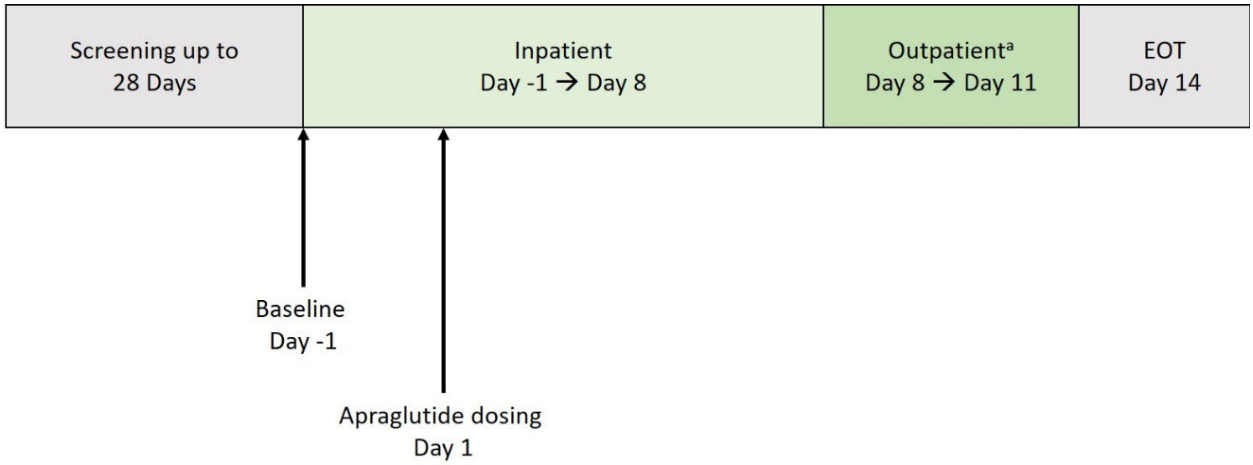
Subjects with mild hepatic impairment (Child-Pugh A) and moderate hepatic impairment (Child-Pugh B) will be enrolled. Patients with decompensation, late-stage cirrhosis, and cirrhosis-related complications (Child-Pugh C) will not be included in this trial, as such patients are not envisioned to be treated chronically with apraglutide. A staged approach will be followed in the trial. Part 2 of the trial will only be conducted after evaluation of Part 1 if the geometric mean ratio (GMR) point estimate of apraglutide AUC_{inf} or AUC_{last} for the moderate hepatic impairment group compared to the control group is ≥2. If this criterion is not met, the trial will stop after Part 1. Subjects will be selected and categorized into normal hepatic function, or hepatic impairment groups based on their Child-Pugh score (will be described in section 9.2.2 of the SAP).

Subjects withdrawn or discontinued from treatment in the normal, mild, or moderate hepatic impairment groups and are considered to be non-evaluable, with respect to the primary PK objective, can be replaced at the discretion of the Sponsor.

Following consent, subjects will undergo a Screening procedure to determine their suitability for trial enrollment. Screening will occur within a 28-day window prior to dosing. Subjects will be admitted on Day -1. On Day 1 a single SC injection of 5 mg apraglutide will be administered. The subjects will remain at the Clinical Research Unit (CRU) post-dose, and either will be discharged on Day 11 or will be allowed to leave the center on Day 8 and will be asked to come back on Day 11 for an outpatient visit. Pharmacokinetics will be assessed on Days 1 to 8, Day 11 and Day 14. The EOT visit will take place on 14±2 days. Pharmacokinetics of blood samples will be assessed for 312 hours (until Day 14 inclusive). Subjects who received apraglutide (except subjects who exit from the trial early) will return to the CRU approximately 14±2 days after dosing for the EOT visit where follow-up assessments will be performed according to the Schedule of Assessments (Appendix 2) and to determine if any AEs have occurred since the last trial visit.

Please find the trial scheme below:

Figure 1: Trial scheme



[a] Some subjects will remain at the CRU and will be discharged on Day 11 while some others will be allowed to leave the CRU on Day 8 and will be asked to return for an outpatient visit on Day 11.

6.1 Sample Size Considerations

No formal power calculation was performed. The number of subjects per group is based on a review of the literature, and a review of the European Medicines Agency (EMA) and Food and Drug Administration (FDA) guidelines.

The sample size of approximately eight subjects per group is also based on the feasibility to recruit subjects with various degrees of hepatic impairment.

6.2 Randomization

The study is not randomized.

7.0 Overview of Planned Analysis

7.1 Changes from Protocol

There are no changes from the protocol.

7.2 Interim Analysis and Key Results

An interim PK report will be created by the pharmacokineticist.

After Part 1 the PK results will be sent by the PK lab to the pharmacokineticist. This will be sent per Data Transfer Agreements created by ICON Data management and will contain Quality controlled (QC'd) results. Based on these results the pharmacokineticist will calculate the required PK Parameters for the interim analysis based on nominal sampling time points. The PK parameters required for the interim reporting will be AUC_{inf} (or AUC_{last}) and C_{max}.

The interim report will contain the raw concentration data, a table with at least the primary PK parameters and a table showing the GMR point estimate of apraglutide AUC_{inf} (or AUC_{last}) and C_{max} for the moderate hepatic impairment group compared to the control group.

If the AUC_{inf} is extrapolated by more than 20% for 1 subject, the AUC_{last} should be used for all subjects for the determination of the Geometric Mean Ratio (GMR). If the GMR for the moderate hepatic impairment group versus the normal group is ≥2.0, Part 2 may be conducted.

7.3 Final Analysis

Draft TFLs will be provided after database lock. After Sponsor comments have been incorporated, the TFLs will be finalized and incorporated in the first draft CSR.

8.0 Data Review

8.1 Data Management

Data handling and transfer will take place under the ICON Data Management Plan for the study.

8.2 Acceptance of Data for Summarization

Programming of analysis datasets and tables, figures and listings (TFLs) may be ongoing during the data management of the study. However, programming of analysis datasets and TFLs will be completed and quality controlled (QC'd) after database lock. Only quality assured (QA'd) results released by the Safety Laboratory, Bioanalytical Laboratory, or other external data source will be used for the programming of analysis datasets and TFLs for the final report. Any data values requiring investigation or corrections that are identified while programming the analysis datasets and TFLs will be sent to the project Data Manager. If the issue affects the TFLs the Programmer or Statistician who identified the issue will follow it to resolution.

9.0 Definitions and General Analysis Methods

9.1 Analysis Data Presentation

9.1.1 Rounding

In listings, data will be presented with the same precision as the original data. Derived data will be rounded for presentation purposes.

For all summaries, the mean and median will be presented to one decimal place greater than the data, standard deviation to two greater than the data, and the minimum and maximum will be presented to the same number of decimal places as the data. Frequency percentages will be presented with one decimal. Except for 100, which will be presented as an integer

The above rule can be applied directly to collected data. For derived data (e.g. the mean values of triplicates ECG's) rounding will occur for presentation purposes

PK concentration and parameters will be presented to three significant figures except %CV (which will be presented with 1 decimal) and t_{max} (which will be presented with 2 decimals). PK Parameter values >1000 will be presented as an integer.

P-values will be reported to four decimal places; p-value less than 0.0001 will be reported as p <0.0001.

9.1.2 Imputation

Unless otherwise noted, data will not be imputed.

Except for the substitution of any PK concentrations below the lower limit of quantification (LLOQ) (see Section 16.2.1), and missing severity and relationship classification, start or end date/times of Adverse Events (AEs) for the calculation of onset and duration (see Section 17.1.1), and the imputation for laboratory results as stated below, any missing data will not be imputed.

Laboratory Data (LB) that are <x or >x (eg "<1.03", ">1000"): the analysis value or reference range value will be the value of the detection limit itself plus or minus one precision unit for the parameter concerned (respectively 1.02 and 1001 in the example). The values before imputation ("<1.03", ">1000") will only be shown in listings.

Imputations / substitutions will be used in calculations and summaries only. Original data will be listed.

9.1.3 Descriptive Statistics

Unless otherwise indicated, continuous variables will be summarized with the following descriptive statistics: n (number of observations), (arithmetic) mean, standard deviation (SD), minimum (min) value, median, and maximum (max) value. PK concentrations and parameters will additionally be summarized with CV, Geometric Mean, and Geometric CV.

Categorical data will be summarized with frequencies and percentages. Percentages by categories will be based on the number of subjects exposed within a treatment.

For categorical data the categories will be presented in the tables exactly as they appear in the CRF / Database.

9.1.4 Pooling

No Data pooling will be performed.

9.1.5 Unscheduled Measurements

Unscheduled measurements will be included in the listings. With the exception of unscheduled measurements used for baseline, unscheduled measurements will be excluded from the descriptive statistics and statistical analysis.

In case an unscheduled measurement was performed immediately after the scheduled measurement because of a previous measurement error (e.g. equipment failure), this repeated measurement will be used, and the original erroneous measurement will be excluded from the analysis.

9.2 Analysis Data Definitions

9.2.1 Baseline Definition

Unless otherwise stated, baseline for post-dose evaluations is defined as the last observation recorded before the study drug administration. The last observation can be an unscheduled / repeated measurement.

9.2.2 Treatment/Subject Grouping

Assignment of subjects to a hepatic function group will be based on the Child-Pugh score at Screening.

Label	Grouping
IMP	Apraglutide (TA799): a single 5 mg dose of apraglutide administered by subcutaneous (SC) injection to the abdomen.
Hepatic Function Group	Cohort 1: moderate hepatic impairment (Child-Pugh B score 7 – 9 points) Cohort 2: normal hepatic function Cohort 3: mild hepatic impairment (Child-Pugh A score 5 – 6 points)

The Child-Pugh score will be determined as follows:

Child-Pugh Score	Point Scored for observed Findings		
	1 point	2 points	3 points
Encephalopathy grade*	0	1 or 2	3 or 4
Ascites	Absent	Slight	Moderate
Serum bilirubin (mg/dL)	<2.0	2 – 3	>3
Serum Albumin (g/dL)	>3.5	2.8 – 3.5	<2.8
Prothrombin time, sec prolonged	<4	4 – 6	>6

*Grade 0: normal consciousness, personality, neurological examination, electroencephalogram.
Grade 1: restless, sleep disturbed, irritable/agitated, tremor, impaired handwriting, 5 cps waves.
Grade 2: lethargic, time-disoriented, inappropriate, asterixis, ataxia, slow triphasic waves.
Grade 3: somnolent, stuporous, place-disoriented, hyperactive reflexes, rigidity, slower waves.
Grade 4: unrousable coma, no personality/behavior, decerebrate, slow 2-3 cps delta activity.

9.2.3 Common Variable Derivations

Variable	Data Type	Definition/Calculation
Change from Baseline	All	Post-dose Observation minus Baseline Observation
Analysis Study Day (Prior to Dose)	All (except ADPP)	Date of Measurement minus Dose Date
Analysis Study Day (Post Dose)	All (except ADPP)	Date of Measurement minus Dose Date +1

9.2.4 QC

The analysis datasets and the TFLs will be QC'd according to the PRA QC plan.

9.2.4.1 Critical Data

The QC plan requires datasets to be classified as critical or non-critical. As the primary objective of this study is to characterize the pharmacokinetics the datasets considered critical are subject level, and pharmacokinetic (ADSL, ADPC, and ADPP). As these are related to the primary objectives these datasets will be double programmed per the QC Plan.

Table 2 ADaM Datasets

ADaM Dataset Name	Description
ADSL	Subject-Level Analysis Dataset

ADaM Dataset Name	Description
ADAE	Adverse Events Analysis Dataset
ADEG	ECG Analysis Dataset
ADLB	Laboratory Test Results Analysis Dataset
ADPC	Pharmacokinetic Concentrations Analysis Dataset
ADPP	Pharmacokinetic Parameters Analysis Dataset
ADVS	Vital Signs Analysis Dataset

9.2.5 ADaM Datasets and Metadata

The analysis datasets will be generated in accordance with Clinical Data Interchange Standard Consortium (CDISC) Analysis Data Model (ADaM) Version 2.1.

ADaM compliant datasets will be delivered to the sponsor. A define.xml file version 2 with the corresponding metadata will be included. Analysis results metadata are excluded.

9.3 Software

The statistical analysis and reporting will be done using SAS® for Windows™ Version 9.4 or higher (SAS Institute, Inc.).

PK parameter calculations will primarily be done using Phoenix® WinNonlin® version 8.1 or higher (Certara, USA, Inc.) Additional PK computations may be performed in SAS®.

9.4 Statistical Methods

9.4.1 Statistical Outlier Determination

No statistical outlier analysis is planned.

9.4.2 Predetermined Covariates and Prognostic Factors

There are no predetermined covariates or prognostic factors.

9.4.3 Hypothesis Testing

No formal hypothesis testing will be done.

9.5 TFL Layout

The layout of Tables, Figures and Listings (TFLs) will be according to the ICON IEB (former PRA EDS) standards.

Table shells are provided with and approved as part of this SAP. Small changes to shell layout due to the nature of the data may be required after lock at the discretion of the ICON lead biostatistician. Other changes to the shells may be out of scope. The TFLs will be provided as a single document in Adobe PDF format (in A4 format), and as individual files for each table, figure and listing in Rich Text Format (.rtf).

Format:

- Page size: A4
- Data in listings will be sorted by hepatic function group, subject number and time point.
- Data in tables will be sorted by hepatic function group and time point.

- Column titles will be in title case letters.
- All tables and listings will be in landscape format.
- The following hepatic function group labels will be used in the TFLs:
  - Normal
  - Mild
  - Moderate

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10.0 Analysis Sets

Analyses	Enrolled Set	Safety Set	Pharmacokinetic Concentrations Set	Pharmacokinetic Parameter Set
Disposition Summaries	✓			
Safety Assessments		✓		
Baseline Characteristics		✓	Demographic summary (if not identical to Safety Set)	Demographic summary (if not identical to Safety Set)
PK Concentrations			✓	
PK Parameters				✓

10.1 Enrolled Set

The enrolled set will consist of subjects who signed the ICF for the study. This includes subjects who are considered screen failures. For these subjects a limited amount of data will be included in the Study Data Tabulation Model (SDTM). This set will be used for disposition summaries.

10.2 Safety Set

The Safety Analysis Set (SAS) comprises all subjects who have received at least one dose of IMP (apraglutide).

All safety analyses will be based upon the Safety Analysis Set.

10.3 Pharmacokinetic Concentrations Set

The PK Concentration Set (PKCS) is a subset of subjects in the SAS who have had at least one PK sample collected.

All PK concentration summaries will be conducted using the PKCS.

10.4 Pharmacokinetic Parameter Set

The PK Parameter Set (PKPS) is a subset of subjects in the SAS who have had at least one PK parameter of interest estimated.

All PK parameter summaries and primary analysis will be conducted using the PKPS.

11.0 Subject Disposition

The number and percentage of subjects enrolled, dosed, and members of each analysis set will be presented. The number and percentage of subjects who withdrew from the study prematurely and a breakdown of the corresponding reasons for withdrawal will also be presented for both the dosed subjects and screen failures.

12.0 Protocol Deviations

Protocol deviations will be listed by subject.

13.0 Demographic and Baseline Characteristics

13.1 Demographics

All demographic data collected during the screenings visit will be listed by subject.

Subject demographics will be summarized descriptively for all subjects by hepatic function group and overall. The summary will include the subjects' age (in years), sex, race, ethnicity, weight (in kg), height (in cm), and BMI (in kg/m2). For female subjects the childbearing potential will be listed as well.

Demographics will be summarized for the safety, PK concentrations and PK Parameter analysis sets, if they are different from one another.

13.2 Medical History

Medical history, categorized by preferred term according to the latest version of Medical Dictionary for Regulatory Activities (MedDRA), will be listed by subject.

13.3 Other Baseline Characteristics

Substance use (tobacco and alcohol) will be listed.

The results of drug and alcohol screen will be listed.

Non-compliance to in- or exclusion criteria (if any) will be listed.

The Child-Pugh Assessment Results will be listed.

Serology results will be listed.

SARS-CoV-2 Results will be listed.

Results for pregnancy, FSH and estradiol will be listed (females only, if required)

14.0 Concomitant Medications

Concomitant medication will be listed. Medications with an end date prior to the first dose of study drug will be considered prior medications and will be noted in the listing. If a partial date allows a medication to be considered concomitant it will be categorized as such. Concomitant medications will be coded by using World Health Organization Drug Dictionary.

15.0 Treatment Compliance and Exposure

Exposure data will be listed by subject.

16.0 Pharmacokinetic Analyses

16.1 Pharmacokinetic Variables

PK concentrations will be collected in plasma and will be analyzed by Altasciences Bioanalysis and Research Services.

16.1.1 Plasma Variables

16.1.1.1 Concentrations

- Plasma concentration of apraglutide

16.1.1.2 Parameters

- PK Parameters for apraglutide as defined in Table 3: Plasma Parameters.

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Table 3: Plasma Parameters

Parameter	Description	SAS Programming Notes
C_{max}	Maximum plasma concentration. Observed peak analyte concentration obtained directly from the experimental data without interpolation, expressed in concentration units	Cmax from WNL
AUC_{last}	Area under the concentration-time curve (time 0 to time of last quantifiable concentration).	AUClast from WNL
AUC_{inf}	Area under the plasma concentration-time curve (time 0 to infinity). calculated as: $AUC_{0\text{-}inf} = AUC_{0\text{-}last} + (C_{last}/k_{el})$ where Clast is the observed concentration at the last time point with concentrations above the lower limit of quantificatation (LLOQ).	AUCINF_obs from WNL If AUC_%Extrap_obs >20% then parameter is flagged
$AUC_{0\text{-}168h}$	Area under the plasma concentration-time curve from time 0 to 168 hours post-dose.	AUC0-168 from WNL where partial time =168
t_{max}	Time to maximum plasma concentration. First observed time to reach peak analyte concentration obtained directly from the experimental data without interpolation, expressed in time units.	Tmax from WNL
k_{el} (also denominated as λ_z)	Terminal phase rate constant calculated by linear regression of the terminal log-linear portion of the concentration vs. time curve. Linear regression of at least three points and an adjusted r^2 greater than or equal 0.80 are required to obtain a reliable k_{el}.	Lambda_z from WNL If adjusted Rsq < .80 then parameter is flagged
$t_{1/2}$	Terminal phase half-life expressed in time units. Adjusted r^2 greater than or equal to 0.80 is required to obtain a reliable $t_{1/2}$.	HL_Lambda_z from WNL If adjusted Rsq < .80 then parameter is flagged
CL/F	Apparent total clearance of the drug from plasma after subcutaneous administration	CL_F_obs from WNL
Vz/F	Apparent volume of distribution during the terminal phase after subcutaneous drug administration	Vz_F_obs from WNL

The PK parameters below will be listed only.		
%AUCextra	Percentage of AUC0-inf that is due to extrapolation from the time of last observed concentration to infinity.	AUC_%Extrap_obs from WNL
Adj r^2	Goodness of fit statistic for the loglinear terminal elimination phase of the concentration time profile identified by least squares linear regression and adjusted	Rsq_adjusted from WNL

	for the number of points (minimum of 3, excluding C_{max}) used in the estimation of K_{el}	
Kel_Start	The start time used in the regression for the determination of K_{el}	Lambda_z_lower from WNL
Kel_End	The end time used in the regression for the determination of K_{el}	Lambda_z_upper from WNL
Kel_N	The number of points used in the regression for the determination of K_{el}	No_points_lambda_z from WNL

Note: AUCs will be calculated using linear up / log down, expressed in units of concentration x time.

16.2 Pharmacokinetic Summaries

16.2.1 Pharmacokinetic Concentrations

Plasma concentrations for apraglutide below the quantifiable limit (BQL) will be set to ½ lower limit of quantification (LLOQ) in the computation of mean concentration values. Descriptive statistics (number of subjects, arithmetic mean, geometric mean, standard deviation (SD), coefficient of variation (CV), Geometric CV, median, minimum, and maximum) will be used to summarize the plasma concentrations by hepatic function group at each scheduled time point. If over ½ the subjects in a given cell have values BQL then the descriptive statistics will not be presented and will instead display as BQL for the mean and minimum. With the exception of maximum all other statistics will be missing. The individual plasma concentrations data will be presented together with the descriptive statistics.

Linear and semi-logarithmic plots of the geometric mean (including Error Bars) plasma concentration by scheduled sampling time will be provided by hepatic function group. These plots will show time in hours. The plots will match the summary table results and will not have an observation at a given time point if more than half of the subjects have values BQL.

Linear and semi-logarithmic plots of the individual plasma concentration by actual sampling time will be provided by subject. These plots will show time in hours. Individual plots will use the BQL handling procedure described below for “Pharmacokinetic Parameters”.

Combined individual plasma concentrations both on a linear and semi-logarithmic scale versus actual sampling time in hours, showing all subjects together in a single plot, will be presented by hepatic function group.

16.2.2 Pharmacokinetic Parameters

PK parameters for apraglutide will be estimated using non-compartmental methods with WinNonlin®.

The plasma PK parameters will be estimated from the concentration-time profiles. In estimating the PK parameters, BQL values at the beginning of the profile will be set to zero. BQL values that occur after the first quantifiable point will be considered missing. Values that are embedded between BQLs, or quantifiable values occurring after two or more BQLs, will be set to missing at the discretion of the pharmacokineticist. Actual sampling times, rather than scheduled sampling times, will be used in all computations involving sampling times. If the actual time or dose time is missing, the scheduled time may be substituted in order to calculate the PK parameter.

Descriptive statistics (number of subjects, mean, geometric mean, standard deviation, coefficient of variation, Geometric CV, median, minimum, and maximum) will be used to summarize the calculated PK parameters by hepatic function group. For t_{max}, only median, min and max will be presented. The individual PK parameters will be presented together with the descriptive statistics.

The points to be included in the k_{el} range will be determined by the pharmacokineticist after inspection of the semi-log concentration-time profiles. At least 3 points will be required to be used. The C_{max} data point will not be included.

Parameters based on adjusted r^2 below 0.80 or $\%AUC_{extra}$ above 20% will be flagged but not excluded from descriptive statistics.

Box and whisker plots for individual subject parameters (AUC_{inf} or AUC_{last} and C_{max}) will be constructed by hepatic function group and overlaid with geometric means.

16.2.2.1 Statistical Analyses

Part 1:

Analysis of variance (ANOVA) will be used to compare the natural log transformed AUCinf or AUClast (if AUCinf cannot be calculated) and Cmax for apraglutide between normal hepatic function group (Reference) and the moderate impaired hepatic group (Test).
If there is a dropout in the moderate hepatic function group, the "matched" subject in the normal hepatic function group will be excluded from this analysis. The subject from the normal hepatic group may however be used in in the analysis for part 2 if there is a matched Mild hepatic function group subject.

If the AUCinf is extrapolated by more than 20% for 1 subject, the AUClast should be used for all subjects for the determination of the Geometric Mean Ratio (GMR).
Estimates of the mean differences (Test-Reference) and corresponding 90% CIs will be obtained from the model. The mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the GMR (Test/Reference) and 90% CIs for the ratios.

The following SAS PROC MIXED pseudo-code may be used:

```
proc mixed data = adpp;
   by parameter;
   class hepatic_group;
   model log(aval) = hepatic_group;
   lsmeans hepatic_group / cl alpha = 0.1;
   estimate "Moderate hepatic impairment vs Normal hepatic function"
hepatic_group 1 -1 /e cl alpha=0.1;
run;
```

Part 2 (if conducted):

Part 2 may be conducted if apraglutide AUCinf (or AUClast when AUCinf cannot be calculated) GMR for the moderate hepatic impairment group versus the normal group is ≥ 2.

Analysis of variance will be used to compare the natural log transformed AUCinf or AUClast and Cmax for apraglutide between normal hepatic function group from Part 1 (Reference) and the mild impaired hepatic group (Test). Estimates of the mean differences (Test-Reference) and corresponding 90% CIs will be obtained from the model. The mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the GMR (Test/Reference) and 90% CIs for the ratios.

The following SAS PROC MIXED pseudo-code may be used:

```
proc mixed data = adpp;
   by parameter;
   class hepatic_group;
   model log(aval) = hepatic _group;
   lsmeans hepatic _group / cl alpha = 0.1;
   estimate "Mild hepatic impairment vs Normal renal function" hepatic_group
1 -1 /e cl alpha=0.1;
run;
```

17.0 Safety Analyses

17.1 Safety Variables

The following safety variables will be summarized:

- Adverse Events (AEs)
- Vital Signs
    - Supine Blood Pressure
        - Systolic Blood Pressure (SBP)

        - Diastolic Blood Pressure (DBP)
    - Pulse rate
    - Temperature
- Electrocardiograms (ECG)
    - Heart Rate
    - PR Interval
    - QRS-Duration
    - QT Interval
    - QTc (Frederica) Interval
    - RR Interval
- Clinical Laboratory Evaluations
    - Clinical Chemistry
    - Hematology
    - Hemostasis

17.1.1 Adverse Events

All AE summaries will include only treatment-emergent adverse events. Treatment-emergent adverse events (TEAE) are those which occur or worsen after the dose of study drug.

An overview of AEs with number of events, and numbers and percentages of subjects reporting TEAEs, TEAEs related to study drug (related or unrelated), TEAEs by common terminology criteria for AEs (CTCAE) grade/severity, serious AEs (SAEs), AEs of Special Interest, AEs of Particularly Interest, and subjects who discontinued due to an AE, will be presented by hepatic function group and overall.

A breakdown of the number of events, and number and percentage of subjects reporting each TEAE, categorized by body system and preferred term coded according to the Medical Dictionary for Regulatory Activities (MedDRA) (Version according to the coding conventions), will be presented by hepatic function group and overall. Counting will be done by subject only, not by event; subjects will only be counted once within each body system or preferred term. A similar table for all drug-related TEAEs will be presented per treatments.

A summary of events reported, categorized by relationship to study drug, will be provided and presented by hepatic function group and overall. Subjects with multiple events within a particular body system or preferred term will be counted under the category of their most drug-related event within that body system or preferred term. Relationship will be mapped to related/unrelated based on the CRF categories. TEAEs reported as definitely, probably, and possibly will be considered related. Unlikely and unrelated will be mapped to unrelated.

A summary of events reported, categorized by severity as recorded on eCRF, will be provided and presented by hepatic function group and overall. Subjects with multiple events within a particular body system or preferred term will be counted under the category of their most severe event within that body system or preferred term.

Listings of adverse events of special interest (AESI) (as reported by the investigators) will be provided separately for each type of AESI. Similarly, listings of adverse events of particularly interest (defined in Table 4) will be provided separately for each medical concept.

Table 4 Adverse Events of Particularly Interest

AEPI	Definition
Acceleration of neoplastic growth including Intestinal neoplastic growth/hypertrophic effect	SOC: Neoplasms benign, malignant and unspecified (incl cysts and polyps)

including polyps	
Benign neoplasia of the Gastrointestinal tract including the hepatobiliary system (not including polyps).	SMQ: Gastrointestinal premalignant disorders (Level 1, narrow scope) SMQ: Biliary neoplasms benign (incl cysts and polyps) (Level 3, narrow scope)
Gastrointestinal obstruction	SMQ: Gastrointestinal obstruction (Level 2, Narrow scope)
Gastrointestinal stoma obstruction	PT: Intestinal anastomosis complication, Stoma obstruction
Increase of the liver transaminases	SMQ Drug related hepatic disorders - comprehensive search (Broad and Narrow)
Embryofetal toxicity	SMQ: Pregnancy and neonatal topics (Broad and Narrow)
Biliary adverse events	SMQ: Functional, inflammatory and gallstone related biliary disorders (Broad and Narrow)
Pancreatic adverse events	HLT: Acute and chronic pancreatitis HLT: Pancreatic disorders NEC
Cardiovascular AEs associated with fluid overload	SMQ: Haemodynamic oedema, effusions and fluid overload (Narrow) SMQ: Cardiac failure (Broad and Narrow)

A listing of adverse events leading to study discontinuation will be provided.

All adverse events (including non-treatment-emergent events) recorded on the eCRF will be listed.

The following missing data will be imputed as defined (for calculations only / will not be presented):

- Missing AE start and / or end times for the calculation of onset and duration will be assumed to be at 00:01 for a start time and 23:59 for end times
- Missing AE severity or relationship will be assumed to be severe or related, respectively
- Missing AE start times for the determination of treatment emergence will be assumed to occur after treatment unless partial date documents the AE as happening prior to treatment
- Missing AE start times for the determination of treatment assignment will be assumed to occur after treatment on the recorded date one minute after dosing
- Missing AE start date will be assumed to be after treatment for the determination of TEAE and on treatment for single treatment studies but will not be attributed to treatment in studies with multiple treatments

17.1.2 Deaths and Serious Adverse Events

A listing of deaths and other serious adverse events (SAE) will be provided by subject.

17.1.3 Laboratory Data

Clinical laboratory data will be presented using units from the study data tabulation model (SDTM) Controlled Terminology.

All laboratory data will be listed, including laboratory variables not listed in the protocol. A separate listing, including out-of-range values will also be provided. Normal ranges will be used directly from the clinical laboratory. Clinically significant results will be flagged.

Descriptive statistics summarizing continuous laboratory results of clinical chemistry, hematology and hemostasis (observed and derived changes from baseline) by hepatic function group and scheduled time will be included in the listings.

17.1.4 Vital Signs

Descriptive statistics will be provided to summarize vital signs values (observed and changes from baseline) by hepatic function group and scheduled time/visit and individual vital signs results will be listed.

The arithmetic mean (including Error Bars) of the vital signs parameters (observed and change from baseline) will be plotted over time per Hepatic Function Group.

17.1.5 Electrocardiograms

All ECG parameters (including changes from baseline) and the corresponding abnormalities and physicians' conclusions will be listed by subject.

The observed measurements for all ECG parameters and the corresponding abnormalities will be listed for all timepoints. The means of triplicate measurements for continuous parameters and the change from baseline of the mean triplicate measurements at each scheduled timepoint will be listed by subject.

For ECG, baseline is defined as the mean of the triplicate measurements recorded at the last timepoint before dosing. If no triplicate is available before the first study drug administration, the mean of the last recorded duplicate closest to the first study drug administration will be considered as baseline. If no triplicate or duplicate is available, the last single ECG recorded before the first study drug administration will be considered as baseline.

Descriptive statistics will be provided to summarize mean ECG parameters (observed and changes from baseline) by hepatic function group and scheduled time/visit.

The arithmetic mean (including Error Bars) of the ECG parameters (mean values of the triplicates, observed and change from baseline) will be plotted over time per Hepatic Function Group.

17.1.6 Other Observations Related to Safety

Abnormal findings at physical examinations will be listed.

18.0 References

SAS Institute, Inc., SAS® Version 9.4 software, Cary, NC.

Phoenix WinNonlin® version 8.1 (Certara USA, Inc. Princeton, NJ, USA)

Clinical Study Protocol: A Phase 1, Open-Label Evaluation of the Pharmacokinetics and Safety of a Single Dose of Apraglutide in Subjects with Normal and Impaired Hepatic Function. Version 1.0, Final, 06 Oct 2022.

Appendix 1: Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
ADaM	Analysis data model
ANOVA	Analysis of variance
BMI	Body mass index
BQL	Below the quantifiable limit
CDISC	Clinical Data Interchange Standard Consortium
CI	Confidence interval
CSR	Clinical study report
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDS	Early Development Service
EMA	European Medicines Agency
IEB	Early Clinical & Bioanalytical Services
FDA	Food and Drug Administration
GMR	Geometric Mean Ratio
ICH	The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
LLOQ	Lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetic
QA'd	Quality assured
QC'd	Quality controlled
SAP	Statistical analysis plan
SAE	Serious adverse event
SC	Subcutaneous
SD	Standard Deviation
SDTM	Study data tabulation model
TEAE	Treatment-emergent adverse event
TFL(s)	Tables, figures and listings
WHO-DDE	World Health Organization – Drug Dictionary Enhanced
WNL	WinNonlin

DocUUID : 829c474d-e195-4988-a17e-1e7f90e4acd3

Appendix 2: Schedule of Assessments

Visit Identifier[a]	Screening Visit 1 Day-28 to Day -1	D -1	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 11	14±2[p] days End of Trial/Early Termination
Informed Consent	X											
Instruct Subjects on lifestyle requirements and restrictions	X									X		
Admission to CRU		X										
Medical History	X	X[n]										
Inclusion/Exclusion	X	X	X									
Demography[b]	X											
Physical examination[c]	X	X			X[c]					X		X
Height and weight assessment for BMI[d]	X	X										X
Safety laboratory tests (blood, urine)[e]	X	X		X						X	X	X
Child-Pugh score[f]	X											
Serum pregnancy test (women of child bearing potential only)	X											X
Urine pregnancy test (women of child bearing potential only)		X										
Contraception check[g]	X	X								X		X
Serum estradiol and serum FSH in postmenopausal females, if required	X											
Urine drug and/or alcohol test[h]	X	X										
Triplicate ECG[i]	X	X	X	X						X	X	X
Vital Signs (supine BP, heart rate) and body temperature[j]	X	X	X[o]	X	X	X	X	X	X	X	X	X
Anti-HIV, HBsAgB, and anti-HCV testing	X											
Apraglutide administration			X									
Injection site assessment[k]			X	X	X	X	X	X	X	X		X
Plasma PK for apraglutide[l]			X	X	X	X	X	X	X	X	X	X

Visit Identifier[a]	Screening Visit 1 Day-28 to Day -1	D -1	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 11	14±2[p] days End of Trial/Early Termination
Prior and Concomitant treatment	X	X	X	X	X	X	X	X	X	X	X	X
Discharge from CRU[m]											X	
AEs/AESIs/SAEs monitoring	X	X	X	X	X	X	X	X	X	X	X	X
COVID-19 PCR testing		X										

AE=adverse event; AESI=adverse event of special interest; BMI=body mass index; CRU=clinical research unit; D=day; ECG=electrocardiogram; FSH=follicle-stimulating hormone; HbA1c=glycated hemoglobin; HBsAgB=hepatitis B surface antigen; HCV=hepatitis C virus; HIV=human immunodeficiency virus; PCR=polymerase chain reaction; PK=pharmacokinetics; SAE=serious adverse event; TSH=thyroid stimulating hormone

a. Day relative to start of trial treatment (Day 1)
b. Demographics include age and year of birth
c. Complete physical examination (PE) at Screening and abbreviated PE at Day -1. If a complete PE was not completed at Screening visit, then a complete PE must be done at Day -1. Symptom-driven PE only at Day 3. Complete PE at Follow-up (End of Trial or Early Termination visit)
d. Height to be obtained only at Screening. BMI will be calculated at Screening only. Weight to be obtained at Screening, Day -1 and Day 14
e. Safety laboratory assessments include chemistry, hematology, serology (screening only), and urinalysis (and microscopy, if needed) and will be performed at Screening, on Day -1, Day 2, Day 8, Day 11, and End of Trial or Early Termination visit. At Day -1, the results must have no clinically significant findings as per the Investigator's judgment to allow investigational medicinal product administration on Day 1. An optional HbA1c and TSH will be allowed at Screening, if applicable, per the discretion of the Investigator to confirm stability of concurrent medical conditions
f. To confirm eligibility, participants must correspond to one of the following categories of the Child-Pugh score: mild, moderate, or have a normal hepatic function. The Child-Pugh assessment will only be performed in hepatically impaired subjects. Echography might be performed for small ascites.
g. Confirmation of appropriate use
h. This test may be performed at any other time at the discretion of the Investigator
i. Triplicate 12-lead ECG will be performed after supine rest of 10 minutes at Screening, Day -1, Day 1 (pre-dose), Day 1 (4 hours post dose), Day 2 (24 hours post dose) and again on Day 8, Day 11, and End of Trial or Early Termination visit. The triplicates are performed at 1minute intervals for 3 minutes and the average of triplicates for each parameter is calculated and used for the statistical analyses.
j. Obtain blood pressure and heart rate measurements after at least 5 minutes of rest in a supine position. One repeat measurement may be allowed at the discretion of the Investigator
k. Injection site reaction assessments to be assessed and recorded as an AE if present
l. Pharmacokinetic time points will be as follows: 0 (5 minutes pre-dose), 6, 12, 24, 28, 36, 40, 48, 60, 72, 96, 120, 144, 168, 240 and 312 hours
m. Some subjects will remain at the CRU and will be discharged on Day 11 while some others will be allowed to leave the center on Day 8 and will be asked to come back on Day 11 for an outpatient visit
n. Changes since Screening
o. Vital signs on Day 1 at pre-dose, 1 hour and 4-hour post dose time points. Vital Signs will be obtained within 45 minutes prior to dosing on Day 1
p. PK sampling must be done on Day 14 (312 hours), other procedures can be completed ±2 days

Appendix 3: List of End of Text Outputs

List of End of Text Tables and Figures:		
Output	**Title**	**Population Set**
Section 14.1 – Disposition and Demographic Data		
Table 14.1.1	Summary of Subject Disposition	Enrolled
Table 14.1.2.1	Summary of Demographics	Safety
Table 14.1.2.2	Summary of Demographics	PK Concentration
Table 14.1.2.3	Summary of Demographics	PK Parameter
In case PK concentration and parameter analysis set differ from the safety set, summary of demographics will also be presented for the PK concentration and/or the PK Parameter analysis sets		
Section 14.2 – Pharmacokinetic Data		
Table 14.2.1.1	Individual Values and Descriptive Statistics of Apraglutide Plasma Concentrations by Hepatic Function Group and Timepoint	PK Concentration
Table 14.2.1.2	Individual Values and Descriptive Statistics of Apraglutide Plasma PK Parameters by Hepatic Function Group	PK Parameter
Table 14.2.1.3	Analysis of Variance of Pharmacokinetic Parameters	PK Parameter
Figure 14.2.1.4	Geometric Mean Apraglutide Concentrations versus Time (Linear and Semi-Log Scale)	PK Concentration
Figure 14.2.1.5	Combined Individual Apraglutide Concentrations versus Time by Hepatic Function Group (Linear and Semi-Log Scale)	PK Concentration
Figure 14.2.1.6	Individual Apraglutide Concentrations versus Time (Linear and Semi-Log Scale)	PK Concentration
Figure 14.2.1.7	Box Plot of Plasma Pharmacokinetic Parameters	PK Parameter
Section 14.3 – Safety Data		
14.3.1 Adverse Events		
Table 14.3.1.1	Overview of Treatment-Emergent Adverse Events	Safety
Table 14.3.1.2	Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety

Table 14.3.1.3	Summary of Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term	Safety
Table 14.3.1.4	Summary of Treatment-Emergent Adverse Events by Relationship to Study Drug and Severity	Safety
14.3.2 Listing of Deaths and other Serious Adverse Events		
Table 14.3.2.1	Listing of Deaths and Other Serious Adverse Events	Safety
14.3.3 Narratives of Adverse Events Leading to Discontinuation From the Study		
Table 14.3.3	Not part of TFL – Reserved for Narratives in CSR	Safety
14.3.4 Clinical Laboratory		
Table 14.3.4.1	Listing of Abnormal Laboratory Values	Safety
Table 14.3.4.2	Summary of Clinical Laboratory Results – Clinical Chemistry	Safety
Table 14.3.4.3	Summary of Clinical Laboratory Results – Hematology	Safety
Table 14.3.4.4	Summary of Clinical Laboratory Results – Hemostasis	Safety
14.3.5 Other Safety Parameters		
Table 14.3.5.1	Summary of Vital Signs	Safety
Figure 14.3.5.2	Arithmetic Mean plot of Vital Signs Parameters versus Time by Hepatic Function Group	Safety
Table 14.3.5.3	Summary of 12-Lead Electrocardiogram	Safety
Figure 14.3.5.4	Arithmetic Mean plot of Mean ECG Parameters versus Time by Hepatic Function Group	Safety

List of End of Text Listings:	
Output	**Title**
Section 16.2.1 – Disposition	
Listing 16.2.1.1	Subject Disposition
Listing 16.2.1.2	Eligibility Criteria
Section 16.2.2 – Protocol Deviations	
Listing 16.2.2.1	Protocol Deviations
Section 16.2.3 – Excluded Subjects	

Listing 16.2.3.1	Analysis Sets
Section 16.2.4 – Demographics and Baseline Characteristics	
Listing 16.2.4.1	Subject Demographics
Listing 16.2.4.2	Medical History
Listing 16.2.4.3	Drug and Alcohol Screen
Listing 16.2.4.4	Substance Use
Listing 16.2.4.5	Childbearing Potential
Listing 16.2.4.6	SARS-CoV-2 Results
Listing 16.2.4.7	Serology Results
Listing 16.2.4.8	Child-Pugh Assessment Results
Listing 16.2.4.9	Pregnancy, FSH, and Estradiol Results
Section 16.2.5 – Compliance	
Listing 16.2.5.1	Study Drug Administration
Listing 16.2.5.2	Study Dates
Section 16.2.6 – Response Data	
Listing 16.2.6.1	PK Sampling Time Deviations, including comments
Section 16.2.7 – Adverse Events Data	
Listing 16.2.7.1	Adverse Events
Listing 16.2.7.2	Adverse Events Leading to Withdrawal
Listing 16.2.7.3	Adverse Events of Special Interest
Listing 16.2.7.4	Adverse Events of Particularly Interest
Listing 16.2.7.5	Prior and Concomitant Medications
Section 16.2.8 – Laboratory Data	
Listing 16.2.8.1	Clinical Laboratory Results – Hematology
Listing 16.2.8.2	Clinical Laboratory Results – Chemistry
Listing 16.2.8.3	Clinical Laboratory Results – Hemostasis
Listing 16.2.8.4	Clinical Laboratory Results – Urinalysis
Listing 16.2.8.5	Clinical Laboratory Results – Additional Assessments
Listing 16.2.8.6	Clinical Laboratory Results – Comments
Section 16.2.9 – Other Safety Data	
Listing 16.2.9.1	Vital Signs
Listing 16.2.9.2	12-Lead Electrocardiogram Results
Listing 16.2.9.3	Physical Examination
Listing 16.2.9.4	Body Weight

Document History

Version Date	Modified/Reviewed By	Brief Summary of Changes (if created from a template, include template code)
23-Jan-2023	[redacted]	Initial Creation
03-Feb-2023	[redacted]	Updates made after internal review
03-Mar-2023	[redacted]	Updates made after sponsor review
03-Apr-2023	[redacted]	Finalized SAP