CLINICAL TRIAL 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

Trial Type:	Phase 1
EudraCT:	2022-002687-68
IND:	Not applicable
NCT Number	N/A
Trial Identifier:	TA799-015
Investigational Medicinal Product:	Apraglutide (TA799)
Protocol Version and Date:	Version 1, 06-Oct-2022

Short Title: Apraglutide pharmacokinetics and safety in hepatically-impaired subjects

VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland

SPONSOR SIGNATURE PAGE

The Sponsor has approved the current protocol and confirms hereby to conduct the trial according to the protocol, current version of the World Medical Association Declaration of Helsinki, the most current version of ICH-GCP (E6 R2, November 2016) guidelines and the local legally applicable requirements.

Sponsor Medical Expert Signatory:

[redacted]

Oct 11, 2022
Date

VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland

[redacted]

Oct 10, 2022
Date

VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland

SPONSOR INFORMATION PAGE

Sponsor Contact Information:

VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland

INVESTIGATOR SIGNATURE PAGE

I have read and understood this trial protocol and agree to conduct the trial as set out in this trial protocol, the most current version of the World Medical Association Declaration of Helsinki, ICH-GCP (E6 R2, November 2016), and locally applicable requirements, and the following:

- European Union Regulation No 536/2014
- Any amendments to these regulations
- Local laws and regulations

Since the information in this protocol is confidential, I understand that its disclosure to any third parties, other than those involved in approval, supervision, or conduct of the registry is prohibited. I will ensure that the necessary precautions are taken to protect such information from loss, inadvertent disclosure, or access by third parties.

Investigator Name and Qualifications: _____

Site: _____

Phone number: _____

_____ _____

Investigator Signature Date

TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ADL	Activity of daily living
AE	Adverse event
AESI	Adverse event of special interest
AUC	Area under the curve
AUC_{0-168h}	Area under the curve from time zero to 168 hours after apraglutide administration
AUC_{inf}	Area under the curve to infinity
AUC_{last}	Area under the curve from time zero to the last quantifiable concentration
BPM	Beats per minute
CI	Confidence interval
CL/F	Apparent clearance after extravascular administration
C_{max}	Maximum observed plasma concentration
CPS	Cycles per second
CRO	Contract Research Organization
CRU	Clinical research unit
ECG	Electrocardiogram
eCRF	Electronic case report form
EMA	European Medicines Agency
EOT	End of trial
EU	European Union
FDA	Food and Drug Administration
FSH	Follicle-stimulating Hormone
GCP	Good Clinical Practice
GI	Gastrointestinal
GLP-2	Glucagon-like peptide 2
GMR	Geometric mean ratio
GVHD	Graft-versus-host disease
HBsAgB	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council on Harmonisation
IEC	Independent Ethics Committee
IF	Intestinal failure
IMP	Investigational medicinal product
IRB	Institutional Review Board
ISR	Injection site reaction
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
PD	Pharmacodynamics
PK	Pharmacokinetic
PKCS	Pharmacokinetic concentration set
PKPS	Pharmacokinetic Parameter Set

SAE	Serious adverse event
SAS	Safety analysis set
SBS	Short bowel syndrome
SC	Subcutaneous
$t½$	Terminal half-life
t_{max}	Time of maximum plasma concentration
TSH	Thyroid stimulating hormone
US	United States
V_z/F	Apparent volume of distribution after extravascular administration
λ_z	Terminal elimination rate constant

PROTOCOL SYNOPSIS

Rationale

Apraglutide is a synthetic peptide analog of glucagon-like peptide (GLP)-2, which is under development for the treatment of short bowel syndrome (SBS) and graft-versus-host disease (GVHD). Apraglutide acts as a full agonist at the GLP-2 receptor *in vitro* with potency and selectivity comparable to native GLP-2 [Wiśniewski, 2016]. Recently, apraglutide has been given to patients via subcutaneous (SC) injection.

The Food and Drug Administration (FDA) recommends a pharmacokinetic (PK) study in subjects with impaired hepatic function if hepatic metabolism and/or excretion accounts for a substantial portion (>20% of the absorbed drug) of the elimination of a parent drug or active metabolite. The FDA also recommends a hepatic impairment study, even if the drug and/or active metabolite is eliminated to a lesser extent (<20%) and if its labeling or literature sources suggest that it has a narrow therapeutic range. Finally, if the metabolism of the drug is unknown and other information is lacking to suggest that hepatic elimination routes are minor, the FDA recommends that the drug be considered extensively metabolized by the liver [CIDER, 2003].

For the European Medicines Agency (EMA), a PK study in subjects with impaired hepatic function is recommended when: i) the drug is likely to be used in patients with impaired hepatic function, ii) hepatic impairment is likely to significantly alter the PK (especially metabolism and biliary excretion) of the drug and/or its active metabolites, or iii) a posology adjustment may be needed for such patients taking into account the PK/pharmacodynamic relationship [CPMP/EWP/2339/02, 2005].

Apraglutide is being developed for the treatment of SBS and GVHD. Individuals with SBS often need parenteral support. In many cases, this results in parenteral nutrition-associated liver disease. Parenteral nutrition-associated liver disease refers to liver dysfunction caused by intestinal failure, or inability to digest and absorb nutrients, that occurs in the setting of parenteral nutrition. There are three primary types of parenteral nutrition-associated liver disease: steatosis, cholestasis, and gallbladder sludge/stones. Individuals may have one of these disorders or a combination of the three [Nowak, 2020; Abdu-Wasel, 2014].

Graft-versus-host disease is a common complication following allogeneic hematopoietic stem cell transplantation that typically manifests as injury to the skin, gastrointestinal (GI) mucosa, and liver. Even if the incidence of Stage 3–4 liver GVHD is low (approximately 2%), this is a serious condition [Matsukuma, 2016].

Based on the aforementioned Health Authorities' recommendations, and because apraglutide will likely be given to subjects with impaired liver function, the PK and safety of apraglutide will be assessed in subjects with hepatic impairment to determine if there are any differences in the PK in hepatically impaired subjects compared with subjects with normal hepatic function.

A non-randomized, open-label design was chosen to allocate subjects into different cohorts based on their hepatic function estimated with the Child-Pugh classification. A reduced trial design was chosen, which compares subjects with hepatic impairment to those with normal hepatic function. Subjects with moderate hepatic impairment (Child-Pugh B) will be recruited first, followed by subjects with normal hepatic function. This will allow subjects with normal hepatic function to be enrolled with 1:1 matching. Subjects with normal hepatic function will be matched 1:1 for age (±10 years), BMI (±15%), and sex (1:1) to subjects with moderate hepatic impairment.

A two-part trial was chosen as it evaluates data from Part 1 before progressing to the optional Part 2, which will only be conducted if the exposure to apraglutide is substantially increased in subjects with moderate hepatic impairment (see Section 4.1.1). Part 2 will compare the PK of apraglutide in subjects with mild hepatic impairment (Child-Pugh A) to subjects with normal hepatic function (i.e., the control group) from the Part 1 where possible. As many as possible of the matching controls enrolled into Part 1 will serve as matching controls for Part 2. However, additional subjects with normal hepatic function may be enrolled in order to meet the given matching criteria (see Section 5.2). This approach has the advantage of ensuring safety and tolerability in addition to reducing potential risk and exposure to participating subjects. The separation of the trial into two parts also has the advantage of using the same control subjects (as much as possible) in both parts of the trial, thereby reducing the number of subjects needed to be enrolled and the number of subjects exposed to potential risk.

Objectives

Primary Objective

- Part 1: To assess the PK of apraglutide in subjects with moderate hepatic impairment compared with matched control subjects with normal hepatic function following single 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

Secondary Objective

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

Endpoints

Primary Endpoints

Plasma apraglutide primary PK parameters are:

- 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 ($AUC_{0–168h}$)
- Maximum observed plasma concentration (C_{max})

Pharmacokinetic parameters, $AUC_{0\text{-}inf}$, AUC_{last}, $AUC_{0–168h,}$ and C_{max} will be derived from plasma concentrations via non-compartmental methods.

Secondary Endpoints

Plasma apraglutide secondary PK parameters include:

- 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 ($t_{½}$)

These PK parameters will be derived from plasma concentrations via non-compartmental methods.

Safety:

- 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

Trial 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 tested 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 (Table 2).

Subjects withdrawn or discontinued from treatment in the normal or moderate hepatic impairment groups and 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 before 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 and Day 14 for an outpatient visit. Pharmacokinetics will be assessed on Days 1 to 8, Day 11 and Day 14. The End of Trial (EOT) visit will take place on 14±2 days. Pharmacokinetics in 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 (Table 1) and to determine if any AEs have occurred since the last trial visit. The planned length of the trial is up to 42 ±2 days (from Screening through trial completion) for each enrolled subject.

Trial Population

Number of Subjects

A total of approximately 24 to 32 subjects will be enrolled if the two parts of the trial are performed. In Part 1, a total of approximately 16 subjects will be enrolled; approximately eight subjects with moderate hepatic impairment (Child-Pugh B; Cohort 1) and approximately eight subjects with normal hepatic function (Cohort 2) to ensure at least six evaluable subjects in each group. Subjects with normal hepatic function will be matched 1:1 for age (±10 years), BMI (±15%), and sex (1:1) to subjects with moderate hepatic impairment.

If the decision criterion to proceed to Part 2 is met, approximately eight subjects with mild hepatic impairment (Child-Pugh A; Cohort 3) will be enrolled to ensure at least six evaluable subjects in each group. The matching will be a group matching for age, BMI, and sex, with approximately 50% of the subjects with normal hepatic function (Cohort 2) on both sides of the median for age and BMI of all subjects with hepatic

impairment (Cohorts 1 and 3), and with approximately the same sex distribution of the normal hepatic function subjects (Cohort 2) as compared with all subjects with hepatic impairment (Cohorts 1 and 3). As many as possible of the matching controls enrolled into Part 1 will serve as matching controls for Part 2. Additional subjects with normal hepatic function may be enrolled in order to meet the given matching criteria when the last PK sample of the last subject with hepatic impairment has been taken.

Inclusion Criteria

All Subjects

1. Signed and dated Informed Consent Form (ICF) prior to any trial-mandated procedure
2. Male or female subjects aged from 18 to 75 years at the time of signing the ICF
3. Body mass index range from 18 to 35 kg/m^2 and a body weight of more than 50 kg
4. Women of childbearing potential must agree to practice effective contraception and to use a highly effective method of contraception (see Section 7.1.1) during the trial and for 4 weeks after the EOT visit
5. Postmenopausal women. Postmenopausal status is defined as no regular menstrual bleeding for at least 12 months prior to inclusion. Menopause will be confirmed by a serum estradiol concentration of <20 pg/mL and a serum follicle-stimulating hormone (FSH) level of >40 IU/L
6. Male subjects with a female partner of childbearing potential must commit to practice highly effective methods of contraception (e.g., condom, vasectomy) and abstain from sperm donation during the trial and for 2 weeks after the EOT visit. Their partners, if they are women of childbearing potential, must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit. See Section 7.1.3
7. Able to participate, willing to give written informed consent, and comply with the trial restrictions
8. Negative result of SARS-CoV-2 polymerase chain reaction testing in the morning prior to admission to the CRU (Day -1)

Additional Inclusion Criteria for Subjects with Normal Hepatic Function (Cohort 2)

9. No clinically relevant abnormalities identified by a detailed medical history, full physical examination, measurement of heart rate, 12-lead ECG, and clinical laboratory tests

Additional Inclusion Criteria for Subjects with Impaired Hepatic Function (Cohorts 1 and 3)

10. Diagnosis of cirrhosis (due to parenchymal liver disease), which is confirmed and documented by medical history and at least one of the following: physical examination, hepatic ultrasound, computed axial tomography scan, magnetic resonance imaging, and/or liver biopsy
11. Cohort 1: moderate liver disease (Child-Pugh B) which has been clinically stable (defined as no clinically significant change in disease status) for at least 1 month prior to screening at the discretion of the Investigator
12. Cohort 3: mild liver disease (Child-Pugh A) which has been clinically stable (defined as no clinically significant change in disease status) for at least 1 month prior to screening at the discretion of the Investigator

Exclusion Criteria

All Subjects

1. History of clinically significant GI, bronchopulmonary, neurological, cardiovascular, endocrine, or allergic disease
2. Known hypersensitivity to the investigational medicinal product (IMP), any of their excipients or drugs of the same class
3. If capable of reproduction, unwilling to use an effective form of contraception
4. If a female of child-bearing potential, a positive urine/blood pregnancy test
5. Breast-feeding women
6. Positive urine/blood test for alcohol and drugs of abuse at Screening and on Day -1
7. Use of prohibited medications or herbal remedies as detailed in Section 10.2
8. Known presence or history of intestinal polyps
9. Known presence or history of any type of cancer
10. Pancreatic events such as acute pancreatitis, pancreatic duct stenosis, pancreas infection, and increased blood amylase and lipase (>2.0–5.0 × upper limit of normal range)
11. Participation in an investigational drug or device study within 30 days prior to Screening
12. Donation of blood over 500 mL within 2 months prior to Screening
13. Heavy use of tobacco products (i.e., smokes more than 10 cigarettes per day)
14. Concomitant disease or condition that could interfere with, or for which the treatment of might interfere with, the conduct of the study, or that would, in the opinion of the Investigator, pose an unacceptable risk to the subject in this trial
15. Any intercurrent clinically significant illness in the previous 28 days before Day 1 of this study as determined by Investigator

16. Positive results to human immunodeficiency virus (HIV) antigen/antibody combo, hepatitis A (HAV), hepatitis B surface antigen (HBsAgB), or hepatitis C virus (HCV) tests. Subjects recovered from hepatitis B or C can be enrolled, i.e., they have markers of the infection, but the viral load is undetectable. Subjects with evidence of an acute or chronically active hepatitis B or C infection will be excluded. A subject being anti-HBs positive, with an undetectable viral load and with history of vaccination against hepatitis B are eligible for enrolment.
17. Unwillingness or inability to comply with the study protocol for any other reason

Additional Exclusion Criteria for Subjects with Normal Hepatic Function (Cohort 2)

18. Any clinically relevant abnormal laboratory test results that are not in line with subject with normal hepatic function status at the discretion of the Investigator
19. Supine pulse rate less than 40 beats per minute (bpm) or more than 100 bpm at Screening. Supine systolic blood pressure below 90 mmHg or higher than 140 mmHg. Supine diastolic blood pressure below 45 mmHg or higher than 90 mmHg at Screening

Additional Exclusion Criteria for Subjects with Impaired Hepatic Function (Cohorts 1 and 3)

20. Any clinically relevant abnormal laboratory test results that are not in line with subject with stable liver disease status at the discretion of the Investigator
21. Supine pulse rate less than 40 bpm or more than 100 bpm at Screening. Supine systolic blood pressure below 100 or higher than 170 mmHg at Screening. Supine diastolic blood pressure below 60 mmHg or higher than 100 mmHg at Screening
22. Diagnosis of cholestasis and/or gallbladder sludge/stones
23. History of esophageal bleeding within the last 3 months prior to screening
24. Severe hepatic encephalopathy (Grade >2) or degree of central nervous system impairment which the Investigator considers sufficiently serious to interfere with the informed consent, the conduct, the completion, the results of this trial, or constitutes an unacceptable risk to the subject
25. History of liver transplantation
26. Advanced ascites and ascites which require emptying and/or albumin supplementation within 30 days prior to Day 1, or during the course of the trial, as judged by the Investigator
27. Hemoglobin concentration <100 g/L (10 g/dL)

Investigational Medicinal Product

All subjects will receive a single SC dose of apraglutide (5 mg). Apraglutide is supplied as a freeze-dried powder (12.5 mg) for reconstitution in sterile water prior to SC injection.

Each apraglutide treatment will be given on Day 1, within 30 minutes following a light breakfast.

Statistics

Hypothesis

No formal statistical hypotheses will be tested in the trial.

Sample Size Calculation

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 EMA and 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.

Summary of Planned Analyses

Pharmacokinetic Analyses

Non-compartmental methods will be applied to compute the PK parameters. Plasma concentrations and computed PK parameters will be listed and summarized descriptively. Individual and mean plasma concentration versus time data will be presented graphically.

Part 1 of the Trial

Analysis of variance will be used to compare the natural log transformed AUC_{inf} or AUC_{last} (if AUC_{inf} cannot be calculated) and C_{max} for apraglutide between the normal hepatic function group (Reference) and the moderate hepatic impairment group (Test). Estimates of the mean differences (Test-Reference) and corresponding 90% confidence intervals (CI) will be obtained from the model. The mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of the adjusted geometric means (Test/Reference) and 90% CIs for the ratios.

Part 2 may be conducted if apraglutide AUC_{inf} (or AUC_{last} when AUC_{inf} cannot be calculated) GMR for the moderate hepatic impairment group versus the normal group is $\geq$2.0. Apraglutide is well tolerated up to 56.9 mg SC single dose as well as 28.4 mg SC weekly doses, further demonstrating the large therapeutic margin of apraglutide.

Part 2 of the Trial

Analysis of variance will be used to compare the natural log transformed AUC_{inf} or AUC_{last} and C_{max} for apraglutide between normal hepatic function group from Part 1 (Reference) and the mild hepatic impairment 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.

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.

For summary statistics and median/mean plots by sampling time, the nominal PK sampling time will be used. For individual subject plots by time, the actual PK sampling time will be used.

If Part 2 is executed and data for normal, mild, and moderate impairment groups are available, additional analysis will be performed to assess the relationship among appropriate PK parameters and hepatic function.

Safety Analyses

Adverse Events

Adverse events will be listed and summarized by period of AE onset and AE by grade: severe AE, serious AE (SAE), AE leading to discontinuation, and AE leading to interruption. Adverse event data will be summarized using descriptive statistics for quantitative data. The latest version of Medical Dictionary for Regulatory Activities (MedDRA) will be used for coding AEs body system, and preferred terms within each body system will be used for summaries.

Laboratory Safety Data

Clinical laboratory data will be listed by subject. Descriptive statistics will be used to assess any changes in clinical laboratory results during and following trial treatment administration. Values outside the reference ranges will be highlighted and clinical significance stated.

Vital Signs and Electrocardiogram Data

Vital sign measurements at each time point will be listed by subject. Plots of vital signs data will be provided. Electrocardiogram interval measurements and ECG findings at each time point will be listed by subject. Plots of mean ECG data will be provided.

Descriptive statistics will be used to assess any changes in vital signs and ECG results during and following trial treatment administration.

Schedule of Events

Table 1 Schedule of Events

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

1. INTRODUCTION

1.1. Background

Short bowel syndrome (SBS) is a malabsorption disorder that manifests as a collection of signs and symptoms such as diarrhea, steatorrhea, fluid and electrolyte disturbances, dehydration, malnutrition, and weight loss. Short bowel syndrome typically develops after loss of more than two-thirds of the small intestine, e.g., through surgical resection of the bowel secondary to Crohn's disease, mesenteric vascular complications, trauma, or necrotizing enterocolitis. Short bowel syndrome intestinal failure (IF) is defined by the inability to maintain protein, energy, fluid, electrolyte, or micronutrient balance, and patients with SBS-IF are dependent on intravenous fluids, electrolytes and/or nutrients, together termed parenteral support [O'Keefe, 2006]. Other patients present a less severe form of SBS defined as intestinal insufficiency, characterized by a remnant small intestine of 200 cm or less, a fecal energy excretion of more than 2.0 MJ/day, or consecutive small intestinal resections exceeding 150 cm [Jeppesen, 2000]. Generally, SBS-intestinal insufficiency patients can maintain their protein and energy balance through hyperphagia, but some of them are considered as borderline IF patients with a high risk to require intermittent parenteral support.

Following surgical resection, the remaining small intestine may undergo a process of structural and functional adaptation, which may lead to restoration of intestinal secretion and motility in addition to hypertrophy and hyperplasia of the mucosal surface. In patients with a preserved terminal ileum or colon in continuity, an increase in the endogenous hormonal secretion of the peptide hormones glucagon-like peptide (GLP)-1, GLP-2, and peptide YY following nutrient ingestion may play a role in this adaptation. However, for patients with resection of the ileum and colon, these neuroendocrine feedback mechanisms are disrupted and may be associated with the pathophysiological traits in SBS patients such as accelerated gastrointestinal (GI) motility, hypersecretion, diminished GI blood flow, and disturbed barrier function. Together these observations have led to the development and pharmacological use of GLP-2 in the intestinal rehabilitation and treatment of SBS patients [Jeppesen, 2014].

Glucagon-like peptide 2 is a 33-amino acid peptide derived from posttranslational processing of proglucagon in intestinal L-cells located primarily in the terminal ileum and colon [Billiauws, 2017; Wiśniewski, 2016]. Glucagon-like peptide 2 and GLP-2 receptor agonists act in a highly localized manner in the GI tract to enhance nutrient and fluid absorption, stimulate blood flow, increase intestinal barrier function, and inhibit gastric acid secretion and gastric emptying. One of the main biological actions of GLP-2 is organ-specific stimulation of intestinal growth through increased crypt cell proliferation and decreased apoptosis of the mucosal epithelial cells [Jeppesen, 2014; Billiauws, 2017]. Data from previous clinical trials with native GLP-2 and GLP-2 analogs have demonstrated that GLP-2 supplementation has the ability to increase crypt depth and villus height [Jeppesen, 2001; Jeppesen, 2005; Jeppesen, 2011] and enhance the intestinal absorptive capacity in patients with SBS [Jeppesen, 2001; Jeppesen, 2005; Jeppesen, 2011; Vipperla, 2013].

Apraglutide is a synthetic peptide analog of GLP-2 that is under development for the treatment of SBS and graft-versus-host disease (GVHD). Apraglutide acts as a full agonist at the GLP-2 receptor *in vitro* with potency and selectivity comparable to native GLP-2 [Wiśniewski, 2016].

The aim of this Phase 1 open-label trial is to evaluate the pharmacokinetics (PK) and safety of a single apraglutide dose in subjects with normal and impaired hepatic function. The data from subjects with impaired hepatic function will be compared with subjects with normal hepatic function.

1.1.1. Name and Description of the Investigational Medicinal Product

Apraglutide is a synthetic peptide analog of GLP-2 under development for treatment of SBS-IF and acute GVHD; moreover, it acts as a full agonist at the GLP-2 receptor with *in vitro* potency and selectivity comparable with native GLP-2 [Wiśniewski, 2016].

This peptide is designed to have a longer elimination half-life by resisting cleavage via dipeptidyl peptidase-4, the major GLP-2 peptidase, and increasing plasma protein binding, thereby preventing kidney elimination. The systemic half-life in various animal species and human healthy volunteers is significantly prolonged compared with the native human GLP-2. Apraglutide has, in various animal models, shown a trophic effect on the small intestine and maintained mucosal barrier function. Preliminary results from the two Phase 2 apraglutide clinical trials have shown that weekly administration of apraglutide increases intestinal absorption parameters and/or urinary output (surrogate marker of fluid intestinal absorption) in subjects with SBS. In addition, weekly administration may represent a substantial additional benefit to subjects in terms of reduction in daily burden of therapy, local reactions and improvement of quality of life over daily dosing.

1.1.2. Non-clinical Trials

Non-clinical studies performed with apraglutide showed no off-target toxicity and indicated a favorable profile for clinical development.

In a trial with neonatal piglets with resected ileum and jejuno-colic anastomosis, apraglutide significantly increased small-intestinal weight, villus height, crypt depth, intestine length, and reduced fecal fat and energy losses [Slim, 2019].

In rats, apraglutide induced a greater intestinotrophic effect than teduglutide and glepaglutide using the same doses and dosing intervals, and it showed an extended duration of effect compared with teduglutide [Hargove, 2020].

When administered subcutaneously to healthy animals, apraglutide exhibited a trophic effect that increased the small intestine weight (80–180% increase in Sprague-Dawley rats and 20–80% increase in minipigs across different dose levels and time points), and the large intestine weight was also slightly increased. In animal models of chemotherapy-induced intestinal mucosal injury, where the intestinal mucosa was severely injured by cytotoxic agents such as cytarabine or melphalan, apraglutide

promoted mucosal healing and preserved the physical integrity and barrier function of the small intestine. Clinically, such pharmacological activity translates to the animals in attenuation of body weight loss and reduced mortality (40% of the apraglutide-treated mice survived after Day 9 compared with no mice in the vehicle-treated group; p=0.0134) without affecting the efficacy of the chemotherapy regimen or stem cell transplantation.

In animals, apraglutide protected the intestinal mucosa, a beneficial effect on the bacterial flora (microbiota), which maintained a normal equilibrium amongst the commensal bacterial populations [Norona, 2020]. In acute GVHD animal models, treatment with apraglutide improved animal survival, and histopathological examination of intestinal segments showed less severe morphological effects throughout the intestine (including the colon), suggesting that this GLP-2 analog potentially protects against the mucosal damage induced from transplantation.

A detailed review of non-clinical results is provided in the Investigator's Brochure.

1.1.3. Clinical Trials

To date, 130 subjects participated in nine clinical trials with apraglutide, among whom 108 subjects (74 healthy subjects, 26 subjects with SBS, and eight subjects with severely impaired renal function) received at least one dose of apraglutide. These clinical trials are described below:

Three Phase 1 clinical trials:

- Clinical trial GYM-P3-698, a single-ascending dose and multiple-ascending dose, placebo-controlled clinical trial of apraglutide in healthy adult subjects evaluating safety, tolerability, PK, and pharmacodynamics (PD)
- Clinical trial TA799-002, a multiple-dose parallel arm clinical trial in healthy adult subjects to evaluate the effect of multiple subcutaneous (SC) doses of either apraglutide (1, 5, or 10 mg) or placebo once weekly for 6 weeks on the PD marker citrulline
- Clinical trial TA799-014, a non-randomized, open-label, single-dose trial to evaluate the effect of varying degrees of impaired renal function on the PK, safety, and tolerability of apraglutide administered to trial subjects via SC injection

Two Phase 2 clinical trials designed to assess the safety and tolerability and to demonstrate proof-of-concept for apraglutide in SBS subjects:

- Clinical trial GLY-321-2017, an open-label metabolic balance clinical trial in SBS subjects testing a weekly dose of 5 mg apraglutide in SBS patients
- Clinical trial GLY-311-2017, a dose-exploration clinical trial in SBS-IF subjects testing weekly 5 mg apraglutide against placebo and 10 mg apraglutide in an open-label extension

A Phase 3, placebo-controlled trial (TA799-007) evaluating the efficacy and safety of apraglutide in subjects with SBS-IF and its open label extension (TA799-012) are currently ongoing as well as a metabolic balance trial (TA799-013) but no results are available.

So far, apraglutide has been shown to have a good safety profile and was well tolerated at single doses ranging between 1 mg and 56.9 mg and repeated weekly doses ranging between 1 mg and 28.4 mg. Further details can be found in the Safety section (Section 5.3.1.3) of the Investigator's Brochure.

1.1.4. Trial Conduct

The trial will be conducted in compliance with this trial protocol, the current version of the World Medical Association Declaration of Helsinki, the recent International Council on Harmonisation (ICH) harmonized tripartite guideline regarding Good Clinical Practice (GCP; E6 R2, November 2016), and locally applicable requirements including the following:

- European Union Regulation No 536/2014
- United States Food and Drug Administration Title 21 Code of Federal Regulations
- Any amendments to these regulations
- Local laws and regulations

1.2. Rationale

1.2.1. Rationale for Trial Design

A non-randomized, open-label design was chosen to allocate subjects into different cohorts based on their hepatic function estimated with the Child-Pugh classification. A reduced trial design was chosen, which compares subjects with hepatic impairment to those with normal hepatic function. Subjects with moderate hepatic impairment (Child-Pugh B) will be recruited first, followed by subjects with normal hepatic function. This will allow subjects with normal hepatic function to be enrolled with 1:1 matching. Subjects with normal hepatic function will be matched 1:1 for age (±10 years), BMI (±15%), and sex (1:1) to subjects with moderate hepatic impairment.

A two-part trial was chosen as it evaluates data from Part 1 before progressing to the optional Part 2, which will only be conducted if the exposure to apraglutide is substantially increased in subjects with moderate hepatic impairment (see Section 4.1.1). Part 2 will compare the PK of apraglutide in subjects with mild hepatic impairment (Child-Pugh A) to subjects with normal hepatic function (i.e., the control group) from Part 1 where possible. As many as possible of the matching controls enrolled into Part 1 will serve as matching controls for Part 2. However, additional subjects with normal hepatic function may be enrolled in order to meet the given matching criteria (see Section 5.2). This approach has the advantage of ensuring safety and tolerability in addition to reducing potential risk and exposure to

participating subjects. The separation of the trial into two parts also has the advantage of using the same control subjects (as much as possible) in both parts of the trial, thereby reducing the number of subjects needed to be enrolled and the number of subjects exposed to potential risk.

1.2.2. Rationale for Trial Population

The Food and Drug Administration (FDA) recommends a PK study in subjects with impaired hepatic function if hepatic metabolism and/or excretion accounts for a substantial portion (>20% of the absorbed drug) of the elimination of a parent drug or active metabolite. The FDA also recommends a hepatic impairment study even if the drug and/or active metabolite is eliminated to a lesser extent (<20%) and if its labeling or literature sources suggest that it has a narrow therapeutic range. If the metabolism of the drug is unknown and other information is lacking to suggest that hepatic elimination routes are minor, the FDA recommends that the drug be considered extensively metabolized by the liver (Figure 1)[CIDER, 2003].

For the European Medicines Agency (EMA), a PK study in subjects with impaired hepatic function is recommended when: i) the drug is likely to be used in patients with impaired hepatic function, ii) hepatic impairment is likely to significantly alter the PK (especially metabolism and biliary excretion) of the drug and/or its active metabolites, or iii) a posology adjustment may be needed for such patients taking into account the PK/PD relationship [CPMP/EWP/2339/02, 2005].

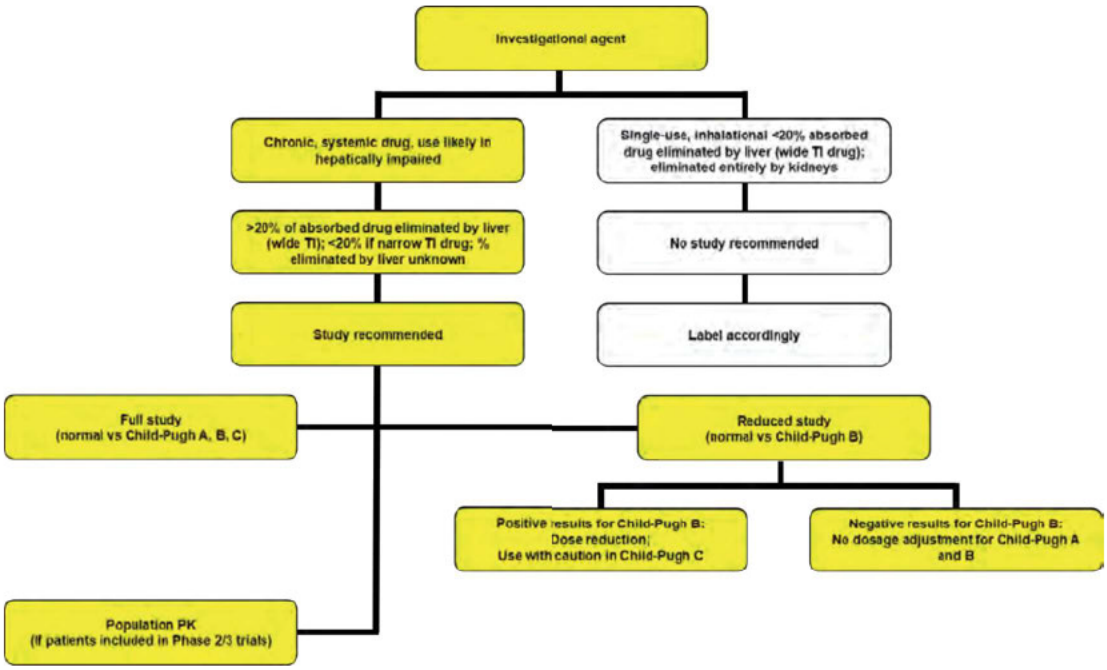
Apraglutide is being developed for treatment of SBS and GVHD. Individuals with SBS often need parenteral support. In many cases this results in parenteral nutrition-associated liver disease. Parenteral nutrition-associated liver disease refers to liver dysfunction caused by IF, or inability to digest and absorb nutrients, that occurs in the setting of parenteral nutrition. There are three primary types of parenteral nutrition-associated liver disease: steatosis, cholestasis, and gallbladder sludge/stones. Individuals may have one of these disorders or a combination of the three [Nowak, 2020; Abdu-Wasel, 2014].

Graft-versus-host disease is a common complication following allogeneic hematopoietic stem cell transplantation that typically manifests as injury to the skin, GI mucosa, and liver. Even if the incidence of Stage 3–4 liver GVHD is low (approximately 2%), this is a serious condition [Matsukuma, 2016].

Based on the aforementioned Health Authorities' recommendations, and because apraglutide will likely be given to subjects with impaired liver function, the PK and safety of apraglutide will be assessed in subjects with hepatic impairment to determine if there are any differences in the PK in hepatically impaired subjects compared with subjects with normal hepatic function.

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 tested in this trial, as such patients are not envisioned to be treated chronically with apraglutide.

Figure 1: Food and Drug Administration Decision Tree for Hepatic Impairment Study



Ph=phase; PK=pharmacokinetic; TI=therapeutic index
Figure adapted from Clinical Pharmacology 1: Phase 1 Studies and Early Drug Development. https://www.fda.gov/media/84920/download

1.2.3. Dose Rationale

All subjects will be dosed with 5 mg apraglutide, injected subcutaneously in the abdomen. This is the anticipated recommended dose for clinical use. A weekly dosage of 5 mg has been used in two completed Phase 2 studies and is being used in the Phase 3 study TA799-007 and its open label extension TA799-012. In a multiple ascending dose study, a weekly dose of 28.4 mg was given and no maximum tolerated dose has been identified.

1.2.4. Rationale for Comparator

Not applicable.

1.3. Potential Risks and Benefits to Human Subjects

Apraglutide has generally been safe and well tolerated in the Phase 1 and 2 clinical trials conducted to date as demonstrated by the following:

- A dose relationship has not been seen for any adverse event (AE) in any of the trials

- Doses up to 56.9 mg were safe and well tolerated in the single-ascending dose and doses up to 28.4 mg in the multiple-ascending dose parts of the Phase 1 trial GYM-P3-698 in healthy volunteers
- Apraglutide dose of 10 mg was well tolerated in healthy volunteers who received up to 6 weekly doses in trial TA799-002
- The most frequent AEs in GLY-311-2017 included nausea, GI stoma output decreased, and stoma complications
- Frequent AEs in GLY-311-2017 were primarily related to the expected efficacy effects of apraglutide, including decreased stoma output or stoma output abnormal, polyuria, and decreased thirst
- Serious AEs (SAEs) have primarily been disease complications that are common in subjects with SBS, including device-related sepsis and device malfunction. Two subjects had a total of three treatment-emergent SAEs. All the SAEs resolved and only one SAE, abdominal pain, was considered by the Investigator as related to apraglutide
- For each hematology, coagulation, and chemistry parameter, mean and median changes from baseline were not clinically significant. Isolated occurrences of clinically significant out of range laboratory parameters were reported as AEs, but there was no consistent pattern with the occurrence of these events
- No QT interval corrected according to Fridericia's formula (QTcF) prolongation greater than 500 milliseconds was seen in any of the clinical trials. In GLY-311-2017 and in GLY-321-2017, the changes from baseline in the QTcF values were ≤30 milliseconds for all subjects at all time points, except for one subject at the End of Trial (EOT) visit and in another subject at pre-dose for Period 3
- Low-titer anti-drug antibodies (ADAs) were seen in five subjects out of 64 treated (none in GYM-P3-698, one in TA799-002, three in GLY-311-2017, and one in GLY-321-2017). The ADAs had no apparent effect on either the PD or PK of apraglutide

In summary, the safety and tolerability profile of a once weekly administration with apraglutide derived from clinical trials conducted so far confirms the expected mode of action-related safety effects.

1.3.1. Assessment of Risk: Benefit Profile

Apraglutide is a novel, synthetic, long-acting GLP-2 analog. Its main role is to increase mesenteric blood flow and to activate pro-absorptive pathways in the gut facilitating nutrient absorption. Additionally, GLP-2 enhances gut barrier function and induces proliferative and cytoprotective pathways in the small bowel. In animal models and in normal animals apraglutide has demonstrated an effect of increased intestinal weight that appears to be the result of both increased intestinal villi and diameter (as expected for a GLP2 analog), as well as increased intestinal length. Pharmaceutical class effects associated with GLP-2 analogs include possible exacerbation of the risk of malignancies, GI obstruction, gallbladder, biliary tract, and

pancreatic disease, and increased absorption of fluids leading to fluid overload in patients with underlying cardiovascular disease.

Up to June 2022, apraglutide has been investigated in healthy volunteers, patients with SBS, and renally impaired patients at doses ranging up to 56.9 mg in the single ascending dose part and doses up to 28.9 mg in the multiple ascending dose part of the Phase 1 clinical trial GYM-P3-698. The safety profile of apraglutide is mainly based on the observed events in patients with SBS at doses from 5 mg to 10 mg. At data cut off, unblinded data of 29 patients with SBS is available as well as 51 patients exposed to apraglutide or placebo. The most frequent AEs were decrease in GI stoma output, stoma complication, nausea, fatigue, injection site erythema, diarrhea, and abdominal pain. All observed Grade 3 or 4 AEs or SAEs were isolated events with the exception of device related sepsis. The only AE currently considered expected, and causally associated with exposure to apraglutide, is abdominal pain. With regards to laboratory assessment, increases in liver transaminases were observed at a higher dose (56.8 mg).

In light of the pharmaceutical class, and observations in the pre-clinical and clinical trial setting, the Sponsor has implemented appropriate assessments and risk management activities such as information in the Informed Consent Form (ICF), Investigator's Brochure and relevant regulatory documents.

Efficacy data from the two Phase 2 apraglutide clinical trials show increased intestinal absorption and/or urinary output in subjects with SBS. Additional benefits to patients receiving weekly administration over daily dosing may include a reduction in the daily burden of therapy, local reactions, and improved quality of life. However, there is no benefit to the subjects participating in this trial.

There are known treatment-related risks reported for other GLP-2 analogs, related to their mode of action, such as the possible risk of accelerating neoplastic growth and enhancing colon polyp growth. Adverse events that have been reported in clinical trials with GLP-2 analogs include injection site reactions (ISRs), abdominal pain, constipation to obstructions, stoma complications, and ileus, biliary, and pancreatic problems [Revestive, SmPC; Gattex®]. Owing to increased absorption, there is also a risk of fluid overload and of overdose with concomitant medications that have a narrow therapeutic window.

Most of these effects were reported after multiple dosing and are unlikely to happen after a single dose.

2. OBJECTIVES

2.1. Primary Objectives

- Part 1: To assess the PK of apraglutide in subjects with moderate hepatic impairment compared with matched control subjects with normal hepatic function following single 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

2.2. Secondary Objective

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

3. ENDPOINTS

3.1. Primary Endpoints

Plasma apraglutide primary PK parameters are:

- 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 ($AUC_{0–168h}$)
- Maximum observed plasma concentration (C_{max})

Pharmacokinetic parameters, $AUC_{0\text{-}inf}$, AUC_{last}, $AUC_{0–168h,}$ and C_{max} will be derived from plasma concentrations via non-compartmental methods.

3.2. Secondary Endpoints

Plasma apraglutide secondary PK parameters include:

- Time of maximum plasma concentration (t_{max})
- Apparent clearance (CL/F)
- Apparent volume of distribution (V_z/F)
- Terminal elimination rate constant (λ_z)
- Terminal half-life (t½)

These PK parameters will be derived from plasma concentrations via non-compartmental methods.

Safety:

- Physical examination
- The incidence, nature and severity of AEs 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

4. TRIAL DESIGN

4.1. Summary of Trial Design

4.1.1. Trial 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 by 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 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 (Table 2).

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 (Table 1) and to determine if any AEs have occurred since the last trial visit.

The trial schematic diagram is shown in Figure 2.

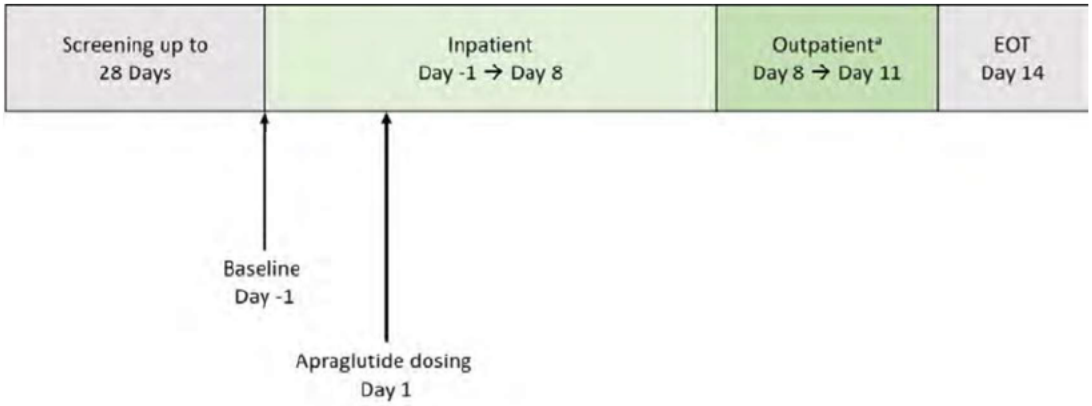
Table 2 Child-Pugh Assessment System

Assessment	Points scored for Observed Findings		
	1	2	3
Encephalopathy grade[a]	none	1 or 2	3 or 4
Ascites	absent	slight	moderate
Serum bilirubin (mg/dL)	<2	2 to 3	>3
Serum albumin (g/dL)	>3.5	2.8 to 3.5	<2.8
Prothrombin time (seconds prolonged)	<4	4 to 6	>6

Child-Pugh assessment scoring system: Child-Pugh A=5 or 6 points; Child-Pugh B=7 to 9 points; Child-Pugh C=10 to 15 points

a. Assessments of encephalopathy should be based on clinical signs and symptoms; an encephalogram is not obligatory for the grading of encephalopathy. Grade 0=normal consciousness, personality, neurological examination, and electroencephalogram. Grade 1=restless, sleep disturbed, irritable/agitated, tremor, impaired handwriting, and 5 cycles per second (CPS) waves. Grade 2=lethargic, time-disoriented, inappropriate, asterixis, ataxia, and slow triphasic waves. Grade 3=somnolent, stuporous, place-disoriented, hyperactive reflexes, rigidity, and slower waves. Grade 4=unrousable coma, no personality/behavior, decerebrate, slow 2–3 CPS delta activity

Figure 2 Trial Schematic Diagram



EOT=end of treatment

a. Some subjects will remain at the Clinical Research Uni and will be discharged on Day 11 while some others will be allowed to leave the Clinical Research Unit on Day 8 and will be asked to return for an outpatient visit on Day 11 and Day 14.

4.1.2. Randomization and Blinding

Not applicable.

4.1.3. Duration of Subject Participation

The planned length of the trial is up to 42±2 days (from Screening through trial completion) for each enrolled subject.

Screening period: Lasts a maximum of 28 days; starts with the full signature (subject and Investigator/delegate) of the ICF and ends with the subject's enrollment or screening failure.

Treatment period: Starts and ends with the administration of a single apraglutide dose.

Post-treatment observation period: Starts on Day 1, the day of dosing of apraglutide, and ends 14±2 days after the dose of apraglutide. For the first 8 days, the subjects will remain at the CRU.

Safety follow-up period: Starts the day after the dose of apraglutide and ends 14±2 days later with the EOT visit. Subjects with AEs related to the investigational medicinal product (IMP) will be followed up until resolution or until deemed unresolvable.

The subject is expected to participate in the trial for a duration of up to 14±2 days.

The visit schedule and protocol-mandated procedures are performed according to the Schedule of Events (Table 1).

4.1.4. Subject Completion

The subject is classified as having completed the trial following the EOT visit.

4.1.5. End of Trial Definition

The end of the trial is defined as the last subject last visit.

The Sponsor may terminate the trial prematurely according to certain circumstances, for example:

- Ethical concerns
- Insufficient recruitment
- When the safety of the subjects is doubtful or at risk, respectively
- Alterations in accepted clinical practice that make the continuation of a clinical trial unwise
- Early evidence of harm of the experimental intervention

4.1.6. Screen Failures

Specific data (demographics, informed consent date, medical history, reason for screen failure and AEs) will be collected during the Screening assessments and entered into the electronic Case Report Form (eCRF) for all subjects that are screen failures.

If after Screening the subject still does not meet all inclusion criteria, or meets one or more of the exclusion criteria, the subject is considered a screening failure. The reason will be entered into the eCRF. If only one laboratory result does not reach the limit for inclusion, one re-testing is allowed within the screening period.

Subjects that failed Screening may be re-screened once at a later stage if the reason for screening failure has changed over time (e.g., out of range laboratory values have resolved). Re-screening must occur at least 1 week from time of screen failure and requires re-consenting and Sponsor approval. Subjects who are re-screened will receive a new subject number.

4.1.7. Trial Safety Supervision

An external expert hepatologist has been appointed to provide ongoing assessments and advice regarding serious hepatic adverse events of special interest (AESI) that require further evaluation during the trial.

4.2. Treatment Alteration and Interruption

Not applicable.

4.3. Stopping Rules

4.3.1. Trial Stopping Rules

The trial can be stopped after completion of Part 1 according to the criterion described in Section 4.1.1.

If the Investigator, the Sponsor, or the Safety Medical Monitor (who reviews medical information and safety data, and consults with the site on eligibility criteria) becomes aware of conditions or events that suggest a possible hazard to subjects if the trial continues, the trial may be terminated after appropriate consultation between the relevant parties. The trial may also be terminated early at the Sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant or unacceptable risk to the subjects enrolled in the trial
- Failure of the site to enroll subjects at an acceptable rate
- Enrollment and further dosing of new subjects will be interrupted if two or more subjects experience AESIs such as fluid overload, persistent abdominal pain, gall bladder, biliary and pancreatic disease of Grade 3 and above, according to the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 5 [CTCAE, 2017] of moderate intensity and/or if one or more subjects experience a Grade 4 event (unless clearly not attributable to the trial drug)

4.3.2. Individual Stopping Rules

This is a single dose trial, therefore, there are no individual stopping rules.

4.4. Subject Withdrawal

4.4.1. Subject Withdrawal from Trial

If a subject is withdrawn from the trial, the Sponsor will be informed immediately, and the subject will be considered an early-termination subject.

If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until the medical situation has resolved.

Regardless of the reason for withdrawal, the Investigator will make every effort to ensure that early-termination subjects who have received any IMP complete the necessary safety follow-up assessments at an EOT visit.

Reason for withdrawal and the date of discontinuation from the trial will be documented under the following categories:

- It is the wish of the subject to withdraw, for whatever reason
- The Investigator judges it necessary due to non-compliance with trial procedures or administrative reasons (including if the subject loses the ability to give consent)
- The Investigator judges it necessary to protect the subject's best interest (including safety concerns related to AEs)
- Medical considerations have emerged including difficulties with obtaining or completing required protocol assessments that, in the opinion of the Investigator, potentially affect the safety of the subject. For example, this may include inability to obtain blood samples for safety assessments
- The subject is pregnant
- The clinical trial is terminated by the Sponsor

4.4.2. Subject Withdrawal from Investigational Medicinal Product

If a subject is withdrawn from the trial, all attempts will be made to ensure a final safety visit (EOT) will be conducted as per the Schedule of Events (Table 1).

For all withdrawn subjects who have been dosed with the IMP, EOT assessments will be performed at an early termination visit performed as soon as possible after the dose. The Investigator will document the date of discontinuation of trial treatment and, if possible, the main reason for the subject's withdrawal or discontinuation in the subject's medical record.

5. TRIAL POPULATION

5.1. Subject Population Description

The subject population will include subjects with hepatic impairment and subjects with normal hepatic function.

5.2. Number of Subjects

A total of approximately 16 to 32 subjects will be enrolled. In Part 1, approximately 16 subjects will be enrolled; approximately eight subjects with moderate hepatic impairment (Child-Pugh B; Cohort 1) and approximately eight subjects with normal hepatic function (Cohort 2) to ensure at least six evaluable subjects in each group. Subjects with normal hepatic function will be matched 1:1 for age (±10 years), BMI (±15%), and sex (1:1) to subjects with moderate hepatic impairment.

If the decision criterion to proceed to Part 2 is met, approximately eight subjects with mild hepatic impairment (Child-Pugh A; Cohort 3) will be enrolled to ensure at least six evaluable subjects in each group. The matching will be a group matching for age, BMI, and sex, with approximately 50% of the subjects with normal hepatic function (Cohort 2) on both sides of the median for age and BMI of all subjects with hepatic impairment (Cohorts 1 and 3), and with approximately the same sex distribution of the normal hepatic function subjects (Cohort 2) as compared with all subjects with hepatic impairment (Cohorts 1 and 3). As many as possible of the matching controls enrolled into Part 1 will serve as matching controls for Part 2. Additional subjects with normal hepatic function may be enrolled in order to meet the given matching criteria when the last PK sample of the last subject with hepatic impairment has been taken.

5.3. Eligibility Criteria

5.3.1. Inclusion Criteria

All Subjects

1. Signed and dated ICF prior to any trial-mandated procedure
2. Male or female subjects aged from 18 to 75 years at the time of signing the ICF
3. Body mass index range from 18 to 35 kg/m^2 and a body weight more than 50 kg
4. Women of childbearing potential must agree to practice effective contraception and to use a highly effective method of contraception (see Section 7.1.1) during the trial and for 4 weeks after the EOT visit
5. Postmenopausal women. Postmenopausal status is defined as no regular menstrual bleeding for at least 12 months prior to inclusion. Menopause will be confirmed by a serum estradiol concentration of <20 pg/mL and a serum follicle-stimulating hormone (FSH) level of >40 IU/L

6. Male subjects with a female partner of childbearing potential must commit to practice highly effective methods of contraception (e.g., condom, vasectomy) and abstain from sperm donation during the trial and for 2 weeks after the EOT visit. Their partners, if they are women of childbearing potential, must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit. See Section 7.1.3
7. Able to participate, willing to give written informed consent, and comply with the trial restrictions
8. Negative result of SARS-CoV-2 polymerase chain reaction testing in the morning prior to admission to the CRU (Day -1)

Additional Inclusion Criteria for Subjects with Normal Hepatic Function (Cohort 2)

9. No clinically relevant abnormalities identified by a detailed medical history, full physical examination, measurement of heart rate, 12-lead ECG, and clinical laboratory tests

Additional Inclusion Criteria for Subjects with Impaired Hepatic Function (Cohorts 1 and 3)

10. Diagnosis of cirrhosis (due to parenchymal liver disease), which is confirmed and documented by medical history and at least one of the following: physical examination, hepatic ultrasound, computed axial tomography scan, magnetic resonance imaging, and/or liver biopsy
11. Cohort 1: moderate liver disease (Child-Pugh B) which has been clinically stable (defined as no clinically significant change in disease status) for at least 1 month prior to screening at the discretion of the Investigator
12. Cohort 3: mild liver disease (Child-Pugh A) which has been clinically stable (defined as no clinically significant change in disease status) for at least 1 month prior to screening at the discretion of the Investigator

5.3.2. Exclusion Criteria

All Subjects

1. History of clinically significant GI, bronchopulmonary, neurological, cardiovascular, endocrine, or allergic disease
2. Known hypersensitivity to the IMP, any of their excipients or drugs of the same class
3. If capable of reproduction, unwilling to use an effective form of contraception
4. If female of child-bearing potential, a positive urine/blood pregnancy test
5. Breast-feeding women
6. Positive urine/blood test for alcohol and drugs of abuse at Screening and on Day -1

7. Use of prohibited medications or herbal remedies as detailed in Section 10.2
8. Known presence or history of intestinal polyps
9. Known presence or history of any type of cancer
10. Pancreatic events such as acute pancreatitis, pancreatic duct stenosis, pancreas infection, and increased blood amylase and lipase (>2.0–5.0 × upper limit of normal range)
11. Participation in an investigational drug or device study within 30 days prior to Screening
12. Donation of blood over 500 mL within 2 months prior to Screening
13. Heavy use of tobacco products (i.e., smokes more than 10 cigarettes per day)
14. Concomitant disease or condition that could interfere with, or for which the treatment of might interfere with, the conduct of the study, or that would, in the opinion of the Investigator, pose an unacceptable risk to the subject in this trial
15. Any intercurrent clinically significant illness in the previous 28 days before Day 1 of this study as determined by Investigator
16. Positive results to human immunodeficiency virus (HIV) antigen/antibody combo, hepatitis A (HAV), hepatitis B surface antigen (HBsAgB), or hepatitis C virus (HCV) tests. Subjects recovered from hepatitis B or C can be enrolled, i.e., they have markers of the infection, but the viral load is undetectable. Subjects with evidence of an acute or chronically active hepatitis B or C infection will be excluded. A subject being anti-HBs positive, with an undetectable viral load and with history of vaccination against hepatitis B are eligible for enrolment
17. Unwillingness or inability to comply with the study protocol for any other reason

Additional Exclusion Criteria for Subjects with Normal Hepatic Function (Cohort 2)

18. Any clinically relevant abnormal laboratory test results that are not in line with subject with normal hepatic function status at the discretion of the Investigator
19. Supine pulse rate less than 40 beats per minute (bpm) or more than 100 bpm at Screening. Supine systolic blood pressure below 90 mmHg or higher 140 mmHg. Supine diastolic blood pressure below 45 mmHg or higher than 90 mmHg at Screening

Additional Exclusion Criteria for Subjects with Impaired Hepatic Function (Cohort 1 and 3)

20. Any clinically relevant abnormal laboratory test results that are not in line with subject with stable liver disease status at the discretion of the Investigator
21. Supine pulse rate less than 40 bpm or more than 100 bpm at Screening. Supine systolic blood pressure below 100 or higher than 170 mmHg at Screening. Supine diastolic blood pressure below 60 mmHg or higher than 100 mmHg at Screening
22. Diagnosis of cholestasis and/or gallbladder sludge/stones

23. History of esophageal bleeding within the last 3 months prior to screening
24. Severe hepatic encephalopathy (Grade >2) or degree of central nervous system impairment which the Investigator considers sufficiently serious to interfere with the informed consent, the conduct, the completion, the results of this trial, or constitutes an unacceptable risk to the subject
25. History of liver transplantation
26. Advanced ascites and ascites which require emptying and/or albumin supplementation within 30 days prior to Day 1, or during the course of the trial, as judged by the Investigator
27. Hemoglobin concentration <100 g/L (10 g/dL)

6. TRIAL ASSESSMENTS AND PROCEDURES

Trial procedures and the times for each assessment are presented in the Schedule of Events Table (Table 1).

The Investigator, or a person designated by the Investigator, will obtain the written ICF from each subject before any trial-specific activity. Subjects will be provided with abundant time to read and ask questions about the ICF. Subjects will also be provided with a copy of the signed and dated ICF.

6.1. Screening Procedures

Subjects will be screened within 28 days prior to administration of apraglutide to confirm that they meet the subject selection criteria for the trial. The following assessments will be completed during Screening (Table 1):

- Informed consent
- Instruct subjects on lifestyle requirements and restrictions
- Obtain medical history, including history of prior recreational drug, alcohol, and tobacco use
- Obtain complete medication history for all prescription or non-prescription drugs, and dietary and herbal supplements taken within 28 days prior to the planned dose
- Collect demography (subject race, ethnicity, birth year, age, sex)
- Conduct a full physical examination; this must be performed by trained medical personnel at the CRU
- Collect height and weight
- Collect blood and urine samples
- Determine Child-Pugh score
- Conduct serum and urine pregnancy test for women of childbearing age
- Contraception check

- Obtain serum estradiol and FSH in postmenopausal females with amenorrhea ≥12 months and under 60 years of age who are not using hormonal contraception or hormone replacement therapy
- Urine drug and/or alcohol test
- Collect 12-lead ECG in triplicate after supine rest of 10 minutes or per CRU standard operating procedure
- Obtain a supine blood pressure and heart rate after at least 5 minutes of rest in a supine position. Repeat measurements may be allowed for safety reasons at the discretion of the Investigator
- Obtain body temperature
- Collect blood for HIV, HBsAg, HB core, and HCV core Ab testing
- Safety laboratory tests
- Adverse event and SAE monitoring

6.2. Efficacy Procedures

Not applicable.

6.3. Pharmacokinetic Procedures

Plasma samples will be collected to determine concentration of apraglutide. Samples will be collected for 312 hours post-dose administration. The specific times for all PK samples are detailed in Table 1. For post apraglutide PK sampling, up to 20 mins post dose, a time window of ±1 minute is permitted. Thereafter, a time window of ±5% of elapsed time post apraglutide dosing is permitted. Outside these time points a protocol deviation should be recorded

Pharmacokinetics will not be assessed for metabolites because:

- No metabolites have known or suspected activity
- No metabolites have been identified as toxic in pre-clinical studies, which could be affected by hepatic function
- No metabolites are suspected to reach active/toxic levels if the accumulation of the metabolites is substantial

Apraglutide will be quantified in human plasma samples using a fully validated liquid chromatography mass spectrometry-based method, with a lower limit of quantification of 1 ng/mL and an upper limit of 200 ng/mL.

At time points when PK blood samples, vital signs, and ECG recordings coincide, the following order is to be maintained: ECG recording, vital signs, and PK sampling.

Samples for PK analysis must always be drawn before administration of apraglutide and the exact time of PK sampling and subsequent apraglutide administration needs to be captured.

Details on blood volume, handling, storage, and shipment of the samples to the central laboratory will be provided in a trial-specific Laboratory Manual. It is important to document the exact time of apraglutide administration and PK sampling.

6.4. Pharmacodynamic Procedures

Not applicable.

6.5. Biomarker and Genetic Assessments

Not applicable.

6.6. Safety Procedures

The specific times for all safety assessments are detailed in Table 1. For post IMP safety assessments, up to 2.5 hours post dose, a time window of ±15 minutes is permitted. For all other time points a time window of ±5% of the elapsed time since doing is permitted.

6.6.1. Physical Examination

A general physical examination is conducted at time points specified in the Schedule of Events (Table 1). This must include height and weight measurement and examination of the eyes, neurological, GI, respiratory, circulatory, muscular, cardiovascular, lymphatic, ears, nose, and throat. An abbreviated physical examination will be conducted on discharge from the CRU. Additional physical examination will be performed at any time during the treatment, if clinically indicated.

This examination serves to detect obvious and severe abnormalities and will be documented in the source documents and eCRF.

6.6.2. Laboratory Assessments

Blood samples for hematology; clinical chemistry, including liver enzymes; and hemostasis and urine samples for urinalysis will be collected at the time points specified in the Schedule of Events (Table 1).

The total blood volume to be collected during this trial will not exceed 200 mL for each subject. For subjects with a positive virology/serology result for hepatitis B and/or C at visit 1, an additional volume, up to a maximum of 4 mL, will be drawn for viral load assessment of hepatitis B and/or C. Repeat or unscheduled samples may be taken for safety reasons or if there were technical issues with a sample.

Blood and urine samples will be processed at the site and sent to the central laboratory for analysis. Details of handling and shipping will be described in the Laboratory Manual. Information of the clinical laboratories involved in the trial will be available in the Laboratory Manual and Site File.

All laboratory reports will be reviewed by the Investigator and parameters out of normal range assessed as "clinically significant" or "not clinically significant." Clinically significant abnormal results during the trial will be appropriately followed up by the Investigator and recorded as an AE.

The following parameters will be assessed during the trial.

6.6.2.1. Hematology

- Erythrocytes
- Hematocrit
- Hemoglobin
- Leucocytes, automated differential counts and percentages
- Thrombocytes (platelets)

6.6.2.2. Clinical Chemistry

- Sodium
- Potassium
- Magnesium
- Chloride
- Calcium
- Glucose
- Creatinine
- Uric acid
- Total bilirubin
- Direct bilirubin
- Triglycerides
- Cholesterol
- Alkaline phosphatase
- Creatinine phosphokinase
- Alanine aminotransferase
- Aspartate aminotransferase
- Albumin
- Total protein
- Gamma-glutamyl transferase
- Lipase

- Amylase

6.6.2.3. Urinalysis

A midstream, clean-catch urine specimen will be collected for dipstick analysis of:

- pH
- Leukocytes
- Protein
- Glucose
- Hemoglobin (blood)

Microscopic analysis will only be performed if results are positive or strong positive for blood, protein, or leukocytes.

6.6.2.4. Additional Parameters

Hemostasis

- Prothrombin time
- International normalized ratio (prothrombin time)

Serology (Virology)

- Human immunodeficiency virus (HIV antigen/antibody)
- Hepatitis A (anti-HAV total)
- Hepatitis B (anti-HBc and HBsAgB)
- Hepatitis C (anti-HCV)

Assessment of Viral Load

For subjects with a positive virology/serology result for hepatitis B or C, viral load will be assessed via polymerase chain reaction (hepatitis B virus DNA or HCV RNA) to ensure that viral load is undetectable.

COVID-19 Testing

All subjects will be required to undergo COVID-19 testing prior to entry to the CRU as per the Investigator site standard operating procedures. Please see Section 13.9.

Pregnancy Test

A serum pregnancy test will be performed for all pre-menopausal female subjects as detailed in the Schedule of Events (Table 1). To be considered sterilized or infertile, women must have undergone surgical sterilization (bilateral tubectomy, hysterectomy or bilateral ovariectomy) or be post-menopausal (defined as at least 12 months amenorrhea without an alternative medical cause; may be confirmed with an estradiol and FSH test if there is doubt). Any pregnancies must be reported.

Drugs of Abuse/Alcohol

Drugs of abuse will be measured in urine/serum. These will include but are not limited to cannabinoids, amphetamines, opiates, methadone, cocaine, benzodiazepines, and barbiturates.

Alcohol will be measured using breath testing.

6.6.3. Twelve-lead Electrocardiogram

Triplicate 12-lead resting ECG will be recorded at the time points specified in the Schedule of Events (Table 1) using the standard equipment at the site. The procedure will be performed with the subject in a supine position after 10 minutes rest. Additional ECG monitoring will be performed at any time during the treatment, if clinically indicated. All ECG parameters, including intervals, (QRS and QT will be recorded. QT corrected according to Fridericia's formula [QTcF]) will be recorded in the eCRF.

Additionally, an overall clinical assessment of the ECG will be made and recorded as "normal," "abnormal not clinically significant," or "abnormal clinically significant." Abnormal clinically significant findings at Screening will be recorded as medical history and clinically significant new findings or worsening in ECG results during the trial must be recorded as an AE.

6.6.4. Vital Signs

Vital signs including systolic and diastolic blood pressure, heart rate, and temperature will be measured at the time points specified in the Schedule of Events (Table 1).

Systolic and diastolic blood pressure will be measured in supine position (after 5 minutes rest). Remaining vital signs will be measured in a supine position after at least 5 minutes rest. Blood pressure will be recorded in mmHg and temperature will be recorded in either centigrade or Fahrenheit.

6.7. Patient-reported Outcomes

This trial is not investigating efficacy; therefore, this is not applicable.

6.8. Pregnancy

See Section 11.4.

7. LIFESTYLE AND/OR DIETARY RESTRICTIONS

The following restrictions will apply during the trial:

- Alcoholic beverages and/or alcohol containing products are not allowed 48 hours before admission to the CRU until the last scheduled evaluation and blood sample collection before discharge
- Drugs of abuse are not permitted for the entire duration of the trial
- Smoking is not permitted in the CRU. Outside smoking is permitted, but not exceeding 10 cigarettes (or equivalent) per day

7.1. Contraception Requirements

7.1.1. Women of Childbearing Potential

7.1.1.1. Definition of Childbearing Potential

A woman is considered to be of childbearing potential, i.e., fertile, following menarches and until becoming post-menopausal unless permanently sterile.

Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

Post-menopausal state is defined as 12 consecutive months with no menses without an alternative medical cause (ICH M3 definition).

7.1.1.2. Methods of Contraception

Women of childbearing potential must agree to practice effective contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit.

Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Vasectomized partner, if this is their sole sexual partner

Women who do not engage in heterosexual intercourse will be allowed to join the trial without contraception following a thorough discussion with the Investigator to determine if this is feasible for the subject. The following methods are not considered acceptable methods of contraception: calendar, ovulation, symptothermal, post-ovulation methods, withdrawal (coitus interruptus), spermicides only, and the lactational amenorrhea method.

7.1.2. Women of Non-childbearing Potential

To be considered sterilized or infertile, females must have undergone surgical sterilization (bilateral tubectomy, hysterectomy, or bilateral ovariectomy) or be post-menopausal (defined as at least 12 months amenorrhea without an alternative medical cause; this will be confirmed with estradiol and FSH tests).

7.1.3. Male Subjects

Male subjects with a female sexual partner of childbearing potential must commit to practice highly effective methods of contraception (e.g., condom, vasectomy) and abstain from sperm donation during the trial and for 2 weeks after the EOT visit.

Nevertheless, their partners, if they are women of childbearing potential, must agree to practice contraception and to use a highly effective method of contraception during the trial and for 4 weeks after the EOT visit. Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable)
- Intrauterine device
- Intrauterine hormone-releasing system

8. INVESTIGATIONAL MEDICINAL PRODUCT(S)

8.1. Dosage and Administration

All subjects will receive a single SC dose of apraglutide (5 mg). Each apraglutide treatment will be given on Day 1, within 30 minutes following a light breakfast.

8.2. Maintaining the Blind

No blinding procedures are necessary for this open-label trial.

8.3. Treatment Assignment

Once eligibility has been confirmed, the subject will be enrolled. In Part 1, subjects will be assigned to either Cohort 1 (moderate hepatic impairment) or Cohort 2 (normal hepatic function) depending on hepatic function. In Part 2, subjects will be assigned to Cohort 3 (mild hepatic impairment). All subjects will receive 5 mg apraglutide.

8.4. Packaging and Labeling

Packaging and labeling of the IMP is performed by the Clinical Trial Supply vendor in accordance with Good Manufacturing Practice and applicable regulatory requirements.

The freeze-dried apraglutide powder is filled in 2 mL vials, sealed with rubber stoppers and aluminum caps. The subject boxes and individual vials will be labeled according to Annex 13, EudraLex Volume 4 [EudraLex, 2010] and national regulatory requirements.

The CRU will be provided with enough supply for all subjects randomized in the trial. Details are included in the Pharmacy Manual.

8.5. Preparation

Reconstitution and preparation of the solution for SC administration will be performed using aseptic techniques following all applicable local guidelines. Guidelines detailing specific instructions for IMP handling will be made available in a Pharmacy Manual that will be provided to the site.

For reconstitution, sterile water will be injected into the vial to obtain a sterile solution. The vial will be gently swirled until its contents are completely dissolved and the contents of the vial verified to be free of foreign particles.

After reconstitution, the IMP must be injected within a maximum of 2 hours from completing reconstitution to ensure sterility is maintained.

The solution can be drawn up into the syringe immediately following reconstitution and kept at room temperature until administration within 2 hours of reconstitution, or the syringe can be drawn up just before administration. The required amount of reconstituted apraglutide (0.2 mL for 5 mg) will be drawn from the vial into the syringe for SC injection. After drawing up the syringe, it will be inspected for foreign particles and used if judged acceptable for administration.

8.6. Handling and Storage

The Investigator must ensure that the IMP (apraglutide) is stored between 2–8°C in a secure location with controlled access when at the site. At the site, the temperature will be monitored at least once per hour, and recorded in a temperature log as per the policies and guidelines of the site and prior to IMP dispensation. Temperature deviations outside the allowed range must be reported and evaluated prior to use of the IMP. Details will be provided to the site in a Pharmacy Manual.

Based on results from ongoing stability studies, the IMP has a shelf-life of 36 months when stored at 5±3°C. The shelf life may be extended as more data become available.

8.7. Product Accountability and Assessment of Compliance

The Investigator is responsible for maintaining records of all IMP vials and sterile water for injection ampoules received and administered to subjects (IMP accountability). The amount of unused IMP at site will be verified by a Monitor during the trial against used vials. If the Monitor's verification against used vials is restricted by site standard operating procedures, then this verification will be performed by the Monitor using site records in accordance to site-specific IMP destruction policies.

Any discrepancies between the amount of IMP (including sterile water) received and administered must be documented.

The IMP administration time and site of administration (injection) performed at the site will be documented in the subject's medical record.

Compliance will be assessed by recording the injected volume in the IMP accountability log and, unless restricted by the site standard operating procedures, will be verified by a Monitor during the trial against used vials. Compliance will also be assessed during IMP accountability.

8.8. Return and Destruction

All used IMP vials and water for injection ampoules are to be accounted for and kept at site for final reconciliation by the Monitor before being returned or destroyed at the end of the trial according to the Pharmacy Manual. If such final reconciliation by the Monitor is restricted by site standard operating procedures, then this reconciliation will be performed in accordance with site-specific IMP destruction policies. Details are described in the Pharmacy Manual.

8.9. Treatment of Investigational Medicinal Product Overdose

Apraglutide will be administered as a single SC injection by trained trial staff. An overdose is not anticipated. Apraglutide has been tested in humans in a range between 2.5 mg and 56.9 mg. No maximum tolerated dose has been established. If overdose is suspected, the Investigator must treat the subject as appropriate and inform the Sponsor according to specified guidelines (Section 11.3).

8.10. Occupational Safety

No specific risks have been identified for staff handling the IMP. However, standard precautions should be taken to avoid needle stick injuries. The Material Safety Data Sheets will be provided to the Investigator where required by local regulations or is available upon request from the Sponsor.

9. NON-INVESTIGATIONAL MEDICINAL PRODUCTS

Not applicable.

10. CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

Any prior or concomitant treatments will be reported in the CRF along with their daily dosage, duration, and reasons for administration. Concomitant drug and non-drug treatment will be collected. All subjects will be questioned about concomitant treatment at each clinic visit. Treatments taken within 28 days of the first period of treatment will be documented as prior treatment.

Subjects who have received any concomitant treatment may be withdrawn from the trial at the discretion of the Investigator.

10.1. Permitted Medications

No new medication should be started during the trial, unless medically necessary and prescribed by the Investigator or another qualified designee involved in the subject's care and being aware of the subject's participation in the trial.

10.2. Prohibited Medications

- Growth hormone, glutamine or growth factors such as GLP-1, GLP-2 or analogs within the last 3 months prior to Screening and throughout the trial
- Systemic corticosteroids, methotrexate, cyclosporine, tacrolimus, sirolimus, infliximab, or other biologic therapy/immune modifiers within 30 days of Screening and throughout the trial. A dose of up to 1-2 g/kg/day of methylprednisolone or equivalent corticosteroid is allowed
- Citrulline supplements, mycophenolate mofetil, and Janus kinase inhibitors are not allowed during the trial
- Use of prescription drugs within 14 days or five half-lives or non-prescription drugs and dietary and herbal supplements within 7 days or five half-lives, if known, (whichever is longer) prior to IMP dosing unless deemed necessary by the Investigator for the treatment of concurrent disease

11. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

11.1. Adverse Events

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

Adverse events will be collected for all subjects from when the ICF is signed until the EOT visit.

11.1.1. Intensity of Adverse Events

The intensity of AEs is graded using the NCI-CTCAE scale (CTCAE, 2017).

Where the event is not specified in the scale, then the following definitions should be used to determine mild, moderate and severe events:

- Grade 1 (Mild): the event may be noticeable to the subject. It does not usually influence daily activities, and normally does not require intervention
- Grade 2 (Moderate): the event may make the subject uncomfortable. Performance of daily activities may be influenced, and intervention may be needed
- Grade 3 (Severe): the event may cause noticeable discomfort and usually interferes with daily activities. The subject may not be able to continue in the trial, and treatment or intervention is usually needed
- Grade 4 (Life-threatening): the subject was at risk of death; this does not apply if the subject might have been at risk of death if the event was more severe
- Grade 5 (Fatal)

A mild, moderate, or severe (Grades 1–3) AE may or may not be serious. Life-threatening and fatal (Grades 4 and 5) are always serious. These terms are used to describe the intensity of a specific event. Medical judgment will be used on a case-by-case basis.

11.1.2. Relationship to Trial Treatment

Both Investigator and Sponsor will assess the causality of the event in relation to the IMP based on the criteria listed in the ICH E2A guidelines:

Investigators must also systematically assess the causal relationship of AEs to IMP/trial treatment (including any other non-IMPs, radiation therapy, etc.) using the following definitions. Decisive factors for assessing the causal relationship of an AE to the IMP include, but may not be limited to, temporal relationship between the AE and the IMP, known side effects of IMP, medical history, concomitant medication, course of the underlying disease, or trial procedures.

Not related: Not reasonably related to the IMP. Adverse event could not medically (pharmacologically/clinically) be attributed to the IMP under trial in this clinical trial protocol. A reasonable alternative explanation must be available. Adverse events reported as "unlikely" related and "unrelated" will be considered unrelated for reporting purposes.

Related: Reasonably related to the IMP. Adverse event could medically (pharmacologically/clinically) be attributed to the IMP under trial in this clinical trial protocol. Table 3 can be used to differentiate between the AEs related to the IMP.

Adverse events reported as definitely, probably, and possibly related will be considered as "related" for reporting purposes.

Table 3 Assessment of Causality of Adverse Events

	Assessment of Causality				
	Definitely	**Probably**	**Possibly**	**Unlikely**	**Unrelated**
Clearly due to extraneous causes	N	N	N	N	Y
Reasonable temporal association with drug administration	Y	Y	Y/N	N	N
May be produced by subject clinical state, etc.	N	N	N	Y	Y
Known response pattern to IMP	Y/N	Y/N	N	N	N
Disappears or decreases on cessation or reduction in dose	Y	Y/N	N	N	N
Reappears on re-challenge (if possible)	Y	N	N	N	N

IMP=investigational medicinal product; Y=yes; N=no

11.2. Adverse Events of Special Interest

An AESI, serious or non-serious, is an AE of scientific and medical concern specific to the Sponsor's product or program for which ongoing monitoring, additional information, and rapid communication by the Investigator to the Sponsor can be appropriate. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., regulators) might also be warranted in line with Council for International Organizations of Medical Sciences standards and local regulations.

The following are considered AESIs for this trial and must be communicated by the Investigator to the Sponsor within 2 weeks following the event.

11.2.1. Injection Site Reactions

Subjects will be monitored for ISRs for at least 1 hour after IMP administration given at site, during a trial visit at the site, or longer (i.e., until the reaction stops or the subject leaves the site), as necessary. The individual symptoms of the ISR will be reported as AEs throughout the entire duration of the trial.

Data on local tolerability will be collected as an AESI. The following characteristics of ISRs will be documented:

- Pain
- Erythema
- Induration

- Pruritus
- Bruising

Severity (as described in Table 4) and duration of these features of the ISR will be collected by the site by direct observation when the IMP is administered on site.

Table 4 Grading of Severity is based on National Cancer Institute–Common Terminology Criteria for Adverse Events 5.0 in line with other Adverse Events in this trial

Pain	
Grade 1	Mild Pain
Grade 2	Moderate pain; limiting instrumental ADL
Grade 3	Severe Pain; limiting self-care ADL
Pruritus	
Grade 1	Mild, localized reaction with only topical intervention
Grade 2	Moderate with noticeable skin change from scratching (e.g., excoriation). Oral therapy indicated
Grade 3	Widespread and resulting in treatment interruption
Induration	
Grade 1	Mild induration with skin able to slide and pinch up
Grade 2	Unable to pinch up skin but still slides
Grade 3	Severe, unable to slide or pinch, potentially limiting ADL and consideration of treatment interruption
Erythema	
Grade 1	Mild, <2.5 cm
Grade2	Moderate, 2.5–5 cm
Grade 3	>5 cm
Bruising	
Grade 1	Mild, <2.5 cm
Grade 2	Moderate, 2.5–5 cm
Grade 3	>5 cm

ADL=activity of daily living

11.2.2. Gastrointestinal Obstruction

Any GI obstructions will be treated by the Investigator according to the Investigator's judgement and carefully followed up.

11.2.3. Gallbladder, Biliary, and Pancreatic Disease

These will be monitored by symptoms, liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase), bilirubin, lipase, and amylase. Subjects will be treated according to the Investigator's judgement.

11.2.4. Fluid Overload

Subjects will be monitored closely for signs and symptoms related to fluid overload (e.g., edema, due to increased absorption). The Investigator will document cases of substantial fluid overload and manage as per clinical practice for the subject accordingly.

11.2.5. Malignancies

For any subject with a malignancy identified during the trial, a thorough medical history will be taken and documented (e.g., smoking history for lung cancer). Information on type of malignancy, histological type, and grading will be collected. The subject will be closely followed up by the Investigator.

11.2.6. Systemic hypersensitivity

Hypersensitivity and anaphylaxis will be collected as AESIs.

11.3. Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

Medical and scientific judgement will be exercised in deciding whether reporting is appropriate in other situations, such as important medical events or reactions that may not reach the above definition but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These will also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an accident and emergency department or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

All SAEs must be reported immediately and within a maximum of 24 hours to the CRO and/or Sponsor of the trial by using a paper version of the SAE form, which can

be emailed to safetydesk@psi-cro.com or faxed. Local fax-numbers will be made available in the Investigator Site File, if email does not work.

The CRO/Sponsor will re-evaluate the SAE and queries will be raised in the eCRF or by email/fax and returned to the site, requesting clarification or follow-up information if needed. After the initial SAE report, the Investigator is required, proactively, to provide further information regarding the subject's condition. All follow-up information obtained after initially reporting the SAE, updates on the SAE, and any other new SAEs must be entered into the CRF as soon as possible as applicable, i.e., within 24 hours after new information becomes available. The Investigator must update the information as applicable in the eCRF and/or send an email to: safetydesk@psi-cro.com. Local fax-numbers will be made available in the Investigator Site File, if email does not work.Serious AEs resulting in death or which are life-threatening will be reported to the Ethics Committee within 7 days.

The minimum data required for a report is:

- Subject ID
- Trial code/protocol number
- Description of SAE or SAE term
- Assessment of causality relationship to IMP
- Name and contact details of person reporting the event

11.4. Pregnancy

Pregnant subjects must immediately be withdrawn from the clinical trial. Any pregnancy during the treatment phase of the trial and within 90 days after discontinuation of trial medication will be reported to the Sponsor within 24 hours. The course and endpoint of the pregnancy should be followed up carefully, and any abnormal endpoint regarding the mother or the child should be documented and reported.

A female subject and/or partner of a female subject of childbearing potential must be instructed to immediately inform the Investigator if she, or their partner, became pregnant during the trial. Pregnancies occurring up to 90 days after the completion of the test drug must also be reported to the Investigator.

If the Investigator becomes aware of a pregnancy occurring in the subject and/or partner of a subject participating in the trial, it should be reported to the Sponsor. The subject and/or partner of a subject should be counselled and followed as described below.

The Investigator should report all pregnancies to the Sponsor within one working day of becoming aware of them. The pregnancy report for a female partner of the male subject must be forwarded within one working day after written consent is obtained from the pregnant partner. The Investigator will make arrangements for the subject and/or partner to be seen by a specialist who can discuss any risks of continuing with

the pregnancy. Monitoring of the subject should continue until 4 weeks after the outcome of the pregnancy is known.

12. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

12.1. Hypothesis Testing

No formal statistical hypotheses will be tested in the trial.

12.2. Sample Size Assumptions

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 EMA and FDA guidelines.

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

12.2.1. Trial Stopping Criteria

Not applicable.

12.3. Statistical Analysis Considerations

12.3.1. Analysis Populations

12.3.1.1. Safety Analysis 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 SAS.

12.3.1.2. Pharmacokinetic Concentration 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.

12.3.1.3. 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.

12.3.2. Key Elements of Analysis Plan

12.3.2.1. Pharmacokinetic Analyses

Non-compartmental methods will be applied to compute the PK parameters. Plasma concentrations and computed PK parameters will be listed and summarized descriptively. Individual and mean plasma concentration versus time data will be presented graphically.

Part 1 of the trial

Analysis of variance will be used to compare the natural log transformed AUC_{inf} or AUC_{last} (if AUC_{inf} cannot be calculated) and C_{max} for apraglutide between the normal hepatic function group (Reference) and the moderate hepatic impairment group (Test). Estimates of the mean differences (Test-Reference) and corresponding 90% confidence intervals (CI) will be obtained from the model. The mean differences and 90% CIs for the differences will be exponentiated to provide estimates of the ratio of the adjusted geometric means (Test/Reference) and 90% CIs for the ratios.

Part 2 may be conducted if apraglutide AUC_{inf} (or AUC_{last} when AUC_{inf} cannot be calculated) GMR for the moderate hepatic impairment group versus the normal group is ≥2.0. Apraglutide is well tolerated up to 56.9 mg SC single dose as well as 28.4 mg SC weekly multiple doses, further demonstrating the large therapeutic margin of apraglutide.

Part 2 of the trial

Analysis of variance will be used to compare the natural log transformed AUC_{inf} or AUC_{last} and C_{max} for apraglutide between normal hepatic function group from Part 1 (Reference) and the mild hepatic impairment 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.

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.

For summary statistics and median/mean plots by sampling time, the nominal PK sampling time will be used. For individual subject plots by time, the actual PK sampling time will be used.

If Part 2 is executed and data for normal, mild and moderate impairment groups are available, additional analysis will be performed to assess the relationship among appropriate PK parameters and hepatic function.

12.3.2.2. Safety Analyses

Adverse Events

Adverse events will be listed and summarized by dose at the onset of the AE and AE sub group (all grade AE, severe AE, SAE, AE leading to discontinuation, AE leading to dose reduction, AE leading to interruption). Adverse event data will be summarized using descriptive statistics for quantitative data. The latest version of Medical Dictionary for Regulatory Activities (MedDRA) will be used for coding AEs body system, and preferred terms within each body system will be used for summaries.

Laboratory Safety Data

Clinical laboratory data will be listed by subject. Descriptive statistics will be used to assess any changes in clinical laboratory results during and following trial treatment administration values outside the reference ranges will be highlighted and clinical significance stated.

Vital Signs and Electrocardiogram Data

Vital sign measurements at each time point will be listed by subject. Plots of vital signs data will be provided. Electrocardiogram interval measurements and ECG findings at each time point will be listed by subject. Plots of mean ECG data will be provided.

Descriptive statistics will be used to assess any changes in vital signs and ECG results during and following trial treatment administration.

12.3.3. Interim Analysis

Not applicable.

12.3.4. Missing, Unused, and Spurious Data

Strategies for handling missing, unused, or spurious data will be specified in the SAP.

12.3.5. Reporting Deviations from the Statistical Plan

Any deviations from the original statistical plan will be reported in the clinical study report (CSR) with a rationale for the deviation.

13. TRIAL ADMINISTRATION

13.1. Ethical Conduct of the Trial

This clinical trial will be conducted in compliance with this protocol and in accordance with the ethical principles stated in the Declaration of Helsinki 1989 version (United States [US] sites) and in its most current version (non-US sites). The

trial will be conducted in accordance with all applicable laws and regulations of the country where the trial is conducted, and in compliance with applicable GCP Guidelines (April 1996 ICH Guidance for Industry E6 GCP [including archiving of essential trial documents] and the Integrated Addendum to ICH E6 [R2]).

13.2. Sponsor and Investigator Responsibilities

13.2.1. Regulatory and Ethical Considerations

Prior to trial initiation, this protocol, the proposed subject information and ICF, and other documents required by current regulations will be submitted to an Independent Ethics Committee (IEC).

A signed and dated statement that this protocol and the ICF have been approved by the IEC must be filed. In accordance with GCP and applicable regulatory requirements, the trial will not start at a site before receiving the respective IEC approval that must be signed and dated.

Before commencing the trial, the "protocol and required submission package" will be submitted to the competent authority and approval must be obtained and made available to the Sponsor.

Investigators are responsible for promptly informing the IEC and the authorities of all protocol amendments, serious adverse reactions, and suspected unexpected serious adverse reactions occurring during the trial that are likely to affect the safety of the subjects or the conduct of the trial. Information on pregnancies occurring during the trial and pregnancy outcomes qualifying as serious are also to be reported to the IEC.

The protocol may not be modified without written approval from the Sponsor. Protocol modifications or changes may not be initiated without prior written IEC approval except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the trial. Such modifications will be submitted to the IEC and written verification that the modification was submitted will be obtained, as per local regulations.

Substantial Amendments are those considered “substantial” to the conduct of the clinical trial and are likely to have a significant impact on, for example, the safety or physical or mental integrity of the subjects, the scientific value of the trial, the conduct or management of the trial or the quality or safety of the trial drug used in the trial. Documentation of IEC approval must be sent to the Sponsor immediately upon receipt.

The constitution of the IEC must meet the requirements of the participating countries and ICH-GCP (R2) guidelines. A list of the IEC members with names and qualifications plus a statement that it is organized according to ICH-GCP(R2) guidelines and the applicable laws must be provided to the Investigator and to the CRO for filing and archiving as per applicable local law.

13.2.2. Investigator Delegation Responsibilities

While delegation of certain aspects of the trial to Sub-Investigators and trial coordinators is appropriate, the Investigator will remain personally accountable for overseeing the trial closely and for ensuring compliance with the protocol and all applicable regulations and guidelines. The Investigator is responsible for maintaining a list of all persons that are/have been delegated trial-related responsibilities (e.g, Sub-Investigators and trial coordinators) and their specific trial-related duties. If the Investigator will delegate all trial site related medical decisions to another qualified physician, their contact information will be available in the Site File.

Investigators will ensure that all persons who are delegated trial-related responsibilities are adequately qualified and informed about the protocol and their specific duties within the context of the trial. Investigators are responsible for providing the Sponsor with documentation of the qualifications, GCP training, and research experience for themselves and their staff as required by the Sponsor, Institutional Review Board (IRB)/IEC, and the relevant governing authorities/IRB/IEC. The Investigators are responsible for keeping an up-to-date Investigator Site File, which includes all required and relevant trial documents.

13.2.3. Financial Disclosure

Investigators and Sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities/IRBs/IECs. Investigators are responsible for providing information on financial interests during the course of the trial and for 1 year after completion of the trial or for a longer period of time if required by local legislation.

13.2.4. Subject Information and Informed Consent

The Investigators will explain to each subject the nature of the trial, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each subject will be informed that the participation in the trial is voluntary and that he/she may withdraw from the trial at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment.

The subject must be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All subjects for the trial will be provided a subject information sheet and a consent form describing the trial and providing sufficient information for the subject to make an informed decision about their participation in the trial. Enough time needs to be given to the subject to ask questions and to decide whether to participate or not.

The formal consent of a subject, using the approved consent form, must be obtained before the subject is submitted to any trial specific procedures.

The subject will read and consider the statement before signing and dating the ICF and will be given a copy of the signed document. The consent form must also be signed and dated by the Investigator (or his/her designee) at the same time as the subject signs, and it will be retained by the Investigator as part of the trial records.

The Investigator must document the informed consent process in the subject's source documents (medical chart).

In the event of substantial changes to the trial or to the risk:benefit ratio, the Investigator will obtain the signed informed consent of subjects for continued participation in the trial using an EC approved amendment to the ICF.

13.2.5. Subject Privacy and Confidentiality

All sites and laboratories or entities providing support for this trial, must comply with the European Union (EU) General Data Protection Regulation No. 2016/679 (adopted 25-May-2018).

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines. The subject's confidentiality and privacy are to be strictly held in trust by the participating Investigators, their staff, and the Sponsor. This confidentiality is extended to testing of biological samples, including biomarker testing, and any future testing in addition to the clinical information relating to the subject. The subject's contact information will be securely stored at each clinical site for internal use during the trial.

After subjects have consented to take part in the trial, the Sponsor and/or its representative reviews their medical records and data collected during the trial. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market apraglutide; national or local regulatory authorities; and the IRB/IEC, which gave approval for the trial to proceed. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number. However, age and birth year may be collected and used to assist the Sponsor to verify the accuracy of the data, for example, to confirm that laboratory results have been assigned to the correct subject.

The results of trials, containing subjects' unique identifying number, relevant medical records, and possibly age and birth year, will be recorded. They may be transferred to, and used in, other countries that may not afford the same level of protection that applies within the countries where this trial is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the trial results, or to answer questions asked by regulatory or health authorities.

Subject research data, which is for purposes of statistical analysis and scientific reporting, will be entered in the eCRF by the trial staff at each site and then transmitted to, and stored at, the Sponsor or its designee. This will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique trial identification number (unique identifier). The key between the personal data and the unique identifier (subject trial number) will be kept at each clinical site and this information will never leave the respective clinical site. The trial data entry and trial management systems used by clinical sites will be secured and password protected. At the end of the trial, all trial databases will be de-identified and archived by the Sponsor or its designee for a minimal period of 25 years.

13.3. Trial Site Closure

At the end of the trial, all trial sites will be closed. The Sponsor may terminate participation of a trial site at any time. Examples of conditions that may require premature termination of a trial site include, but are not limited to, the following:

- Non-compliance with the protocol and/or applicable regulations and guidelines
- Inadequate subject enrollment
- Discontinuation of the trial, as decided by the Sponsor

13.4. Data Handling and Record Keeping/Archiving

13.4.1. Confidentiality and Access to Source Data

All documentation and materials provided by the Sponsor to the investigational site for this trial are to be retained in an Investigator Site File, which will be stored in a secured location and treated as confidential material.

All local legal requirements regarding protection of personal data must be adhered to.

The trial protocol, documentation, data, and all other information generated during the trial will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

13.4.2. Record Keeping/Archiving

Each clinical site will retain in a secured location trial records and documents pertaining to the conduct of this trial and distribution of the IMP, including eCRFs, ICFs, laboratory test results, and medication inventory records, for at least 25 years after completion or discontinuation of the trial, or for the length of time required by relevant national or local health authorities, whichever is longer. Additionally, record retention may be dependent on the reviewing IRB/IEC, institutional policies, respective country legislation, specifications in trial contract, or Sponsor requirements.

The Sponsor is to be informed about any re-location of documents. After the 25-year archiving period, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of VectivBio. Written notification will be provided to VectivBio prior to transferring any records to another party or moving them to another location.

13.4.3. Storage of Biological Material and Related Health Data

Biological samples will be stored until analysis is complete (CSR is final) or per local regulations, whichever is longer. Analysis will not include any genetic testing.

13.4.4. Data Handling

13.4.4.1. Data Collection, Data Transfer Procedure, and Data Access

The Investigator/delegate is responsible for ensuring the accuracy, completeness, and timely reporting of subject's data.

Fully validated electronic data capture will be used to collect eCRF data. The Investigator and site personnel will be trained to enter and edit the data via a secure network, with secure access features (username, password, and ID, hence an electronic password system). A complete electronic audit trail will be maintained. The Investigator/delegate will approve the data (i.e., confirm the accuracy and completeness of the data recorded) using an electronic signature (as per US 21 Code of Federal Regulations Part 11 and/or EU Annex 11).

All subjects will be recorded in the Screening and Enrollment log. Subject numbers will be allocated in chronological order for each study site.

For each subject recruited, regardless of trial treatment initiation, an eCRF must be completed and signed by the Investigator/delegate. This also applies to those subjects who fail to complete the trial.

13.4.5. Database Management and Quality Control

The eCRF must be completed in a timely manner as per eCRF completion guidelines.

While entering the data, the Investigator/delegate will be instantly alerted to data queries by validated programmed checks. Additional data reviews will be performed by the Sponsor personnel or delegate on an ongoing basis to look for unexpected patterns in data and for trial monitoring. Should discrepant data be detected, a query specifying the matter and requesting clarification will be issued and visible to the Investigator/delegate via the eCRF. All electronic queries visible in the system either require a data correction (when applicable) and a response from the Investigator/delegate to clarify the queried data directly in the eCRF, or simply a data correction in the eCRF. The Investigator/delegate must, on request, supply the Sponsor with any required background data from the trial documentation or clinical

records. This is particularly important when errors in data transcription are suspected. In the event of health authority queries, it is also necessary to have access to the complete trial records, provided that subject data confidentiality is protected.

This process will continue until database lock.

Pharmacokinetic samples will be processed through a central laboratory vendor and the results of the enrolled subjects will be electronically sent to the Sponsor/appointed CRO at pre-specified intervals with a final transfer prior to the database lock.

Adverse events and medical history are coded with MedDRA. Medications are coded with the World Health Organization Drug Dictionary.

After the database has been declared complete and accurate, the database will be locked. Any changes to the database after that time may only be made as described in the appropriate Sponsor/CRO Quality System documents. The Sponsor is responsible for ensuring that the Investigator/delegate will have permanent access (either "write" access or "read-only" access) to the site eCRF subject data, until receipt of an electronic copy of the site eCRFs (including the audit trail).

13.4.6. Specification of Source Documents

Source documents provide evidence for the existence of the participating subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's trial site.

Data entered in the eCRF that are transcribed from source documents must be consistent with the source documents and discrepancies must be explained. The Investigator may need to request medical records from other healthcare professionals, if appropriate. Also, current medical records must be available. The use of electronic source data is described in FDA Guidance for Industry – Electronic Source Data in Clinical Investigations [FDA, 2013].

All trial data must be verifiable by source documents. Additionally, the following data entered into the eCRF will be verifiable by source documents in the subject's medical record, or other records, at the trial site, as applicable:

- Details of trial participation (Trial ID and unique identifier)
- Date(s) of subject's informed consent
- Date of each trial visit including signature and/or initials of person(s) conducting the trial visit
- Results of blood tests and other examinations

Information recorded in the eCRF will be supported by corresponding source documentation that is attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data will be traceable, will not obscure the original entry, and will be explained if necessary. Examples of acceptable source documentation

include, but are not limited to, hospital records, clinic and office charts, laboratory notes, and recorded data from automated instruments and memoranda.

Clinical laboratory data required by the protocol will be electronically transferred from the central laboratory to the Sponsor or its designee. Any other clinical laboratory data received from a local laboratory for entry into the eCRF will be considered as source documentation.

Entries into the eCRF will be verified with source documentation by the Monitor. The location of source data for all pertinent data will be defined in the relevant source data location form prior to the start of the trial.

13.5. Quality Assurance and Quality Control

This trial will be performed in compliance with the clinical trial protocol, the Declaration of Helsinki, ICH-GCP (R2) guidelines, and applicable regulatory requirements.

The accuracy, consistency, completeness, and reliability of the trial data produced under this protocol will be assured through quality control and quality assurance activities performed in accordance with the standard operating procedures of the Sponsor or of the Sponsor representative (i.e., CRO). The Investigator agrees, when signing this protocol, to fully cooperate with compliance checks by allowing direct access to all clinical trial related documentation by authorized individuals.

13.5.1. Audits and Inspections

The trial may be subject to audit by the Sponsor or its designee, IRB/IEC, and/or regulatory authority inspections. Audits may be performed to check compliance with ICH GCP (R2) guidelines, and may include:

- Site audits
- (Electronic) Trial Master File/Investigator Site File audits
- Database audits
- Document audits

The Sponsor or its designee may conduct additional audits on a selection of trial sites, requiring access to subject's notes/medical records, trial documentation, and facilities or laboratories used for the trial.

The trial site, facilities, all data (including source data), and documentation will be made available for audit by quality assurance auditors and for IRB/IEC or regulatory authority inspections according to ICH GCP (R2) guidelines. The Investigator agrees to cooperate with the auditor during the visit and will be available to supply the auditor with eCRF or other files necessary to conduct that audit. Any findings will be strictly confidential. Serious breaches or other significant findings will be reported to Health Authorities and/or Ethics Committees/Independent Review Boards as required by applicable laws and regulations.

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

13.5.2. Monitoring

The Sponsor and/or its representative CRO will conduct site visits to Monitor the trial and ensure compliance with the protocol, GCP, and applicable regulations and guidelines. There will be a Clinical Monitoring Plan that will provide details to identify the Monitor of the trial and guidelines for Clinical Research Associates concerning how to monitor the trial.

To ensure data accuracy, completeness, and compliance, monitoring visits will be performed to review source data versus eCRF. The assigned Monitor will visit the Investigator and trial site at periodic intervals and maintain periodic communication. The Investigator agrees to allow the Monitor and other authorized Sponsor personnel access. The Monitor will maintain current personal knowledge of the trial through observation, review of trial records and source documentation, and discussion of the conduct of the trial with the Investigator and staff. While on site, the Monitor will review the following:

- Source documents, directly comparing entries in the Electronic Data Capture system with the source documents
- Consenting procedures
- Investigator Site file (ICH GCP documents)

The Monitor will ask for clarification and/or correction of any noted inconsistencies. (Procedures for correcting eCRF are described in the eCRF completion guideline.) As representatives of the Sponsor, Monitors are responsible for notifying project management of any noted protocol deviations.

All laboratory data from biomarker samples and from future analyses of the residuals of the biomarker samples will be available only to the Sponsor.

By signing the Investigator's Agreement (found at the start of this protocol), the Investigator agrees to meet with the Monitor during trial site visits; to ensure that trial staff are available to the Monitor as needed; and to provide the Monitor access to all trial documentation, if requested. Furthermore, the Investigator agrees to allow the Sponsor to assist the inspectors in their duties, if requested.

13.6. Publication and Dissemination Policy

Data will be reported in a CSR in compliance with the requirements of the current version of ICH E3: "Structure and Content of Clinical study report."

The CSR will be completed within 12 months after the end of the trial and distributed as required by local regulations.

A description of this clinical trial is available on clinical trial registries such as ClinicalTrials.gov and/or the EU Clinical Trials Register clinicaltrialsregister.eu. A summary of results, whether positive, negative, or inconclusive, will be published on ClinicalTrials.gov or other relevant public registry.

The trial results may be published and presented to the public and used for educational purposes. Information that could identify subjects will not be used in any publication or presentation.

All information concerning the Sponsor's operations, patent applications, basic scientific data, and information supplied by the Sponsor or its designee to the Investigator and not previously published, is considered confidential and remains the sole property of the Sponsor. Case report forms also remain the property of the Sponsor. The Investigator agrees to use this information for purposes of trial execution through finalization and will not use it for other purposes without the written consent of the Sponsor.

The information developed in this trial will be used by the Sponsor in connection with the future development of an IMP and thus may be disclosed as required to other clinical Investigators or government regulatory agencies.

The information generated by this trial is the property of the Sponsor who will disclose the trial results in accordance with applicable regulatory requirements and laws. The results of this trial may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor at least 60 days before submission. This allows the Sponsor to protect proprietary information and to provide comments and approval at least 30 days prior to the relevant publication submission deadline. If more than one trial site will be participating, data from individual trial sites must not be published separately.

Authorship will be determined in line with International Committee of Medical Journal Editors authorship requirements.

The publication policy with respect to the Investigator and clinical trial site will be further detailed in a separate document (e.g. clinical site agreement).

13.7. Insurance

The Sponsor will cover this trial by means of an adequate insurance of the participating subjects that will be in place prior to the start of the trial.

As per local regulations, details about the insurance are described in the subject information sheet and ICF. A copy of the insurance statement is filed in the Investigator site file and the subject can request a copy.

13.8. Funding and Support

The trial is Sponsored by VectivBio, who has taken initiative to the conduct of this trial and will enter into a contract with the trial site.

The Sponsor is paying the trial site as per the signed contract. The signed contract details the amount to be paid (including overhead fee, if applicable) to cover site staff work in relation to the trial. Furthermore, the signed contract details that the Sponsor will reimburse the following expenses for trial subjects: travel expenses, meals, and hotel stays (where relevant). All amounts will be paid to a research bank account held by the trial site. Bank account details are specified in the signed contract.

Details on the Sponsor's payment to the site and reimbursement payments for subjects are also given in the Subject Information Letter and ICF.

13.9. Adaptations Due to the Severe Acute Respiratory Syndrome Coronavirus 2

All subjects will be tested for COVID-19 on Day -1 and as per the standard rules at the CRU.

14. REFERENCES

Abdu-Wasel B, Molinari M. Liver Disease Secondary to Intestinal Failure. Biomed Res Int. 2014;2014:968357.

Billiauws L, Bataille J, Boehm V, Corcos O, Joly F. Teduglutide for treatment of adult patients with short bowel syndrome. Expert Opin Biol Ther. 2017;17(5):623-32.

CIDER CIBER. Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling. May 2003.

Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0. 27 November 2017. Available from: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf [accessed 05 April 2022]

CPMP/EWP/2339/02 Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function. 17 February 2005.

EudraLex. The Rules Governing Medicinal Products in the European Union. Volume 4, EU Guidelines to Good Manufacturing Practice, Medicinal Products for Human and Veterinary Use: Annex 13 Investigational Medicinal Products. February 2010. ENTR/F/2/AM/an D(2010) 3374

FDA. Guidance for Industry: Electronic Source Data in Clinical Investigations. September 2013. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/electronic-source-data-clinical-investigations [accessed 04 April 2022]

Gattex® (teduglutide [rDNA origin]), for injection, for subcutaneous use Initial U.S. Approval: 2012. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/203441Orig1s000lbl.pdf [accessed 05 April 2022]

Hargove DM, Alagarsamy S, Croston G, Laporte R, Qi S, Srinivasan K, et al. Pharmacological Characterization of Apraglutide, a Novel Long-Acting Peptidic Glucagon-Like Peptide-2 Agonist for the Treatment of Short Bowel Syndrome. J Pharmacol Exp Ther. 2020;373(2):193-203.

Jeppesen PB, Mortensen PB. Intestinal failure defined by measurements of intestinal energy and wet weight absorption. Gut. 2000;46(5):701-6.

Jeppesen PB, Hartmann B, Thulesen J, Graff J, Lohmann J, Hansen BS, et al. Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. Gastroenterology. 2001;120(4):806-15.

Jeppesen PB, Sanguinetti EL, Buchman A, Howard L, Scolapio JS, Ziegler TR, et al. Teduglutide (ALX-0600), a dipeptidyl peptidase IV resistant glucagon-like peptide 2 analog, improves intestinal function in short bowel syndrome patients. Gut. 2005;54(9):1224-31.

Jeppesen PB, Gilroy R, Pertkiewicz M, Allard JP, Messing B, O'Keefe SJ. Randomized placebo-controlled trial of teduglutide in reducing parenteral nutrition and/or intravenous fluid requirements in patients with short bowel syndrome. Gut. 2011;60(7):902-14.

Jeppesen PB, Pertkiewicz M, Messing B, Iyer K, Seidner DL, O'Keefe SJ, et al. Teduglutide Reduces Need for Parenteral Support Among Patients With Short Bowel Syndrome With Intestinal Failure. Gastroenterology. 2012;143(6):1473-81.

Jeppesen PB. New approaches to the treatments of short bowel syndrome-associated intestinal failure. Curr Opin Gastroenterol. 2014;30(2):182-8.

Kochar B, Herfarth HH. Teduglutide for the treatment of short bowel syndrome – a safety evaluation. Expert Opin Drug Saf. 2018;17(7):733-9.

Matsukuma KE, Wei D, Sun K, Ramsamooj R, Chen M. Diagnosis and differential diagnosis of hepatic graft versus host disease (GVHD). J Gastrointest Oncol. 2016;7(Suppl 1):S21-S31.

Norona J, Apostolova P, Schmidt D, Ihlemann R, Reischmann N, Taylor G, et al. Glucagon-like peptide 2 for intestinal stem cell and Paneth cell repair during graft-versus-host disease in mice and humans. Blood. 2020;136(12):1442-55.

Nowak K, Parenteral Nutrition-Associated Liver Disease. Clin Liver Dis. 2020;15(2):59-62.

O'Keefe SJ, Buchman AL, Fishbein TM, Jeejeebhoy KN, Jeppesen PB, Shaffer J. Short bowel syndrome and intestinal failure: consensus definitions and overview. Clin Gastroenterol Hepatol. 2006;4(1):6-10.

Revestive, INN-teduglutide; European Medicines Agency, European public assessment report (EPAR)/Summary of Product Characteristics (EMEA/H/C/002345); 2019. Available from: https://www.ema.europa.eu/en/documents/product-information/revestive-epar-product-information_en.pdf

Slim GM, Lansing M, Wizzard P, Nation PN, Wheeler SE, Brubaker PL, et al. Novel Long-Acting GLP-2 Analog, FE 203799 (Apraglutide), Enhances Adaptation and Linear Intestinal Growth in a Neonatal Piglet Model of Short Bowel Syndrome with Total Resection of the Ileum. J Parenter Enteral Nutr. 2019;43(7):891-8.

Vipperla K, O'Keefe SJ. Study of teduglutide effectiveness in parenteral nutrition-dependent short-bowel syndrome subjects. Expert Rev Gastroenterol Hepatol. 2013;7(8):683-7.

Wiśniewski K, Sueiras-Diaz J, Jiang G, Galyean R, Lu M, Thompson D, et al. Synthesis and pharmacological characterization of novel glucagon-like peptide-2 (GLP-2) analogs with low systemic clearance. J Med Chem 2016;59(7):3129-39.

15. APPENDICES PROVIDED FOR TRIAL TA799-015

N/A