VectivBio

STARGAZE
Study of Apraglutide in Graft vs Host Disease

Clinical Trial Protocol

A randomized, single-blind trial to evaluate the safety and efficacy of apraglutide in subjects with Grade II to IV (MAGIC) steroid refractory gastrointestinal (GI) acute graft versus host disease on best available therapy

Short Protocol Title: Proof-of-concept trial of apraglutide in GVHD

Trial Type:	Phase 2
EudraCT:	2021-004588-29
IND:	IND 156438
NCT:	NCT 05415410
Trial Identifier:	TA799-101
Sponsor:	VectivBio AG, Aeschenvorstadt 36, 4051 Basel, Switzerland
Investigational Medicinal Product:	Apraglutide (TA799)
Protocol Version and Date:	5.0, FINAL, 27/Mar/2023

Sponsor Signature Page

Trial number	Protocol number TA799-101
Protocol Title	A randomized, single-blind trial to evaluate the safety and efficacy of apraglutide in subjects with Grade II to IV (MAGIC) steroid refractory gastrointestinal (GI) acute graft versus host disease on best available therapy

The Sponsor has approved the current protocol and confirms hereby to conduct the trial as set out in this protocol, the current version of the World Medical Association Declaration of Helsinki, the recent International Council on Harmonisation harmonized tripartite guideline regarding Good Clinical Practice (E6 R2, November 2016) and the local legally applicable requirements, and the following as applicable:

- 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

Sponsor Representative and Medical Expert

Printed name of Sponsor Representative:

Omar Khwaja
Chief Medical Officer
VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland
+41 61 551 3030

Mar 28, 2023	Omar Khwaja	*Electronically signed by: Omar Khwaja Reason: Approver Date: Mar 28, 2023 08:36 GMT+9*
Date	**Signature**	

Statistician

Printed name of Statistician:

Carrie Li
Program statistician
VectivBio AG,
Aeschenvorstadt 36,
4051 Basel,
Switzerland
+41 61 551 3030

Mar 28, 2023	Chih-Lin Li	*Electronically signed by: Chih-Lin Li Reason: Approver Date: Mar 28, 2023 08:47 GMT+2*
Date	**Signature**	

International Coordinating Investigator

I have read and understood this trial protocol and agree to conduct the trial as set out in this trial protocol, the current version of the World Medical Association Declaration of Helsinki, the recent International Council on Harmonisation harmonized tripartite guideline regarding Good Clinical Practice (E6 R2, November 2016) and the local legally applicable requirements, and the following:

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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.

Prof. Dr. Robert Zeiser

Leiter der Abteilung für Tumorimmunologie und Immunregulation

Klinik für Innere Medizin I (Schwerpunkt Hämatologie, Onkologie und Stammzelltransplantation)

Universitätsklinikum Freiburg

Hugstetter Str. 55,

79106 Freiburg,

Germany

Tel.: 0761 270 34580,

Fax.: 0761 270 36580,

robert.zeiser@uniklinik-freiburg.de

Mar 28, 2023

Electronically signed by: Robert Zeiser
Reason: Approver
Date: Mar 28, 2023 15:08 GMT+2

Date **Signature**

Investigator Signature Page*:

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Date (DD/MMM/YYYY)	Signature
Principal Investigator Name and title	
	Printed Name
Site ID	
Phone number	
Address	

*NOTE: This page must be individually signed by each participating Principal Investigator.

TABLE OF CONTENTS

ABBREVIATIONS

ADA	Anti-Drug Antibodies
AE	Adverse Event
AESI	Adverse Event of Special Interest
aGVHD	Acute Graft Versus Host Disease
alloSCT	Allogeneic Hematopoietic Stem Cell Transplantation
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BAT	Best Available Therapy
BSA	Body Surface Area
BSL	Baseline
CA	Competent Authority
CFR	Code of Federal Regulations
cGVHD	Chronic Graft Versus Host Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration Equation
CL/F	Apparent Clearance
CNI	Calcineurin Inhibitor
CR	Complete Response
CRA	Clinical Research Associate
CRO	Contract Research Organization
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Event
CTR	Clinical Trial Report
DILI	Drug-induced Liver Injury
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EOT	End of Trial
EQ-5D-5L	EuroQol-5 Dimension – 5 Level Survey
EU	European Union
EUPI	European Union Prescribing Information
FACT-BMT	Functional Assessment of Cancer Therapy – Bone Marrow Transplantation
FAS	Full Analysis Set
FDA	Food and Drug Administration
FFS	Failure-free Survival

GCP	Good Clinical Practice
GCS	Glucocorticosteroid
GLP	Glucagon-like Peptide
GI	Gastrointestinal
GVHD	Graft Versus Host Disease
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HV	Healthy Volunteers
ICF	Informed Consent Form
ICH	International Council on Harmonisation
ID	Identification
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IND	Investigational New Drug
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISF	Investigator Site File
ISR	Injection Site Reaction
iSRC	Independent Safety Review Committee
ka	Absorption Rate Constant
LBW	Low Body Weight
LFT	Liver Function Test
MAGIC	Mount Sinai Acute Graft Versus Host Disease International Consortium
MAP	MAGIC Algorithm Probability
MedDRA	Medical Dictionary for Regulatory Activities
MH	Medical History
MP	Methylprednisolone
MR	Magnetic Resonance
NCI	National Cancer Institute
NOAEL	No-Observed-Adverse-Effects Level
NRM	Non-Relapse Mortality
OS	Overall Survival
PD	Pharmacodynamic
PK	Pharmacokinetic
POC	Proof-of-concept
PR	Partial Response
PT	Preferred Term
QTcF	QT Interval corrected according to Fridericia's formula
REG3α	Regenerating Islet-Derived Protein 3 Alpha

RUX	Ruxolitinib
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS-COV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SAS	Safety Analysis Set
SBS	Short Bowel Syndrome
SBS-IF	Short Bowel Syndrome with Intestinal Failure
SC	Subcutaneous
SIV	Site Initiation Visit
SmPC	Summary of Medicinal Product Characteristics
SMQs	Standardized MedDRA Queries
SOC	System Organ Class
SOP	Standard Operating Procedure
SR	Steroid Refractory
SS	Systemic Steroids
ST2	Suppression of Tumorigenicity 2
sTM	Soluble Thrombomodulin
SUSAR	Suspected Unexpected Serious Adverse Reaction
TA799	Apraglutide
TEAE	Treatment-emergent Adverse Event
ULN	Upper Limit of Normal
US	United States
USPI	United States Prescribing Information
VAS	Visual Analog Scale
VEGF	Vascular Endothelial Growth Factor
Vz/F	Apparent Volume of Distribution

TRIAL SYNOPSIS

Sponsor	VectivBio AG
Protocol title:	A randomized, single-blind trial to evaluate the safety and efficacy of apraglutide in subjects with Grade II to IV (MAGIC) steroid refractory gastrointestinal (GI) acute graft versus host disease on best available therapy
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EudraCT number:	2021-004588-29
IND number:	IND 156438
NCT Number:	NCT 05415410
Clinical phase:	Phase 2
Background and rationale:	Graft versus host disease (GVHD) develops in 50–70% of patients who undergo allogeneic hematopoietic stem cell transplantation (alloSCT). Graft versus host disease is an inflammatory disorder that leads to tissue damage and ultimately organ failure and is categorized as either acute GVHD (aGVHD) or chronic GVHD (cGVHD). Acute GVHD is most frequent within the first 100 days after transplantation and is the leading cause of non-relapse mortality (NRM) following alloSCT. Acute GVHD most typically affects the skin, gastrointestinal (GI) tract, and liver. The incidence of GI-aGVHD is reported to be between 40% and 50%. Treatment of GI-aGVHD remains unsuccessful in most cases, and the GI tract is involved in virtually all fatal cases of aGVHD. Ruxolitinib (RUX) was approved by the United States Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of patients aged 12 years and older with aGVHD or cGVHD who have inadequate response to corticosteroids or other systemic therapies. Despite this improvement in treatment options, there is still considerable unmet medical need in this population, as a significant number of patients either do not respond to RUX (30–40%) or lose the initial response over time. Apraglutide is a potent glucagon-like peptide (GLP)-2 receptor agonist and acts through a GI targeted regenerative approach that is intestinotrophic with a mode

	of action that improves absorption and enhances gut barrier function. 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 promotes mucosal healing and preserves the physical integrity and barrier function of the small intestine. Clinically, such pharmacological activity translates to animals in attenuating body weight loss and reducing mortality without interfering with the anticancer efficacy of the chemotherapy or with the efficacy of stem cell transplantation. In total body irradiation animal models of aGVHD, treatment with apraglutide improved animal survival and histopathological examination of intestinal segments showed less severe morphological effects through the entire intestine (including the colon) suggesting that this GLP-2 analog has a potential protective effect against the mucosal damage induced by the transplantation. The pharmacological properties of apraglutide suggest this peptide could have a therapeutic benefit in clinical conditions where the intestine is damaged by aggressive anticancer treatments or aGVHD. This view is further supported by evidence in humans with steroid refractory (SR) GVHD of the GI tract: a cohort of six subjects with SR aGVHD, having failed multiple therapies, were given teduglutide (another GLP-2 analog) at a dosage of 0.05 mg/kg body weight once daily for 10 days. Clinical signs of intestinal GVHD improved in all six subjects with a decline in diarrhea frequency. These findings suggest that GLP-2 may be effective in subjects with SR-GVHD, leading to an improvement in clinical symptoms by improving the GI barrier function. Based on this background, apraglutide could open a new avenue of SR GI-aGVHD treatment by using a novel regenerative and non-immunosuppressive approach through GLP-2 signaling, a signaling cascade that may modulate the regeneration of lost enterocyte mass secondary to inflammation by GVHD together with the repair of impaired intestinal barrier function, resulting in improved intestinal absorption. The aim of this randomized, single-blind, proof-of-concept trial is to evaluate safety, tolerability, efficacy, durable overall response, and clinical outcomes of apraglutide in subjects with SR lower GI-aGVHD of the lower GI tract being treated with systemic steroids (SS) and RUX (defined as best available therapy [BAT]). The data will be compared with a historical control.

Trial objectives:	Primary Objective To assess safety and tolerability of apraglutide in subjects with SR lower GI-aGVHD Grade II to IV Mount Sinai aGVHD International Consortium (MAGIC) who are treated with SS and RUX Secondary Objectives To evaluate the overall response rate (partial response [PR] and complete response [CR]) at Day 56, on the lower GI tract MAGIC score, in subjects with SR lower GI-aGVHD Grade II to IV MAGIC that are treated with apraglutide, SS, and RUX compared to SS and RUX alone (BAT) To evaluate the overall response rate (PR and CR) at Days 14, 28, 91, 119, 147, and 182 on the lower GI-tract MAGIC score To evaluate the overall response rate (PR and CR) at Days 14, 28, 56, 91, 119, 147, and 182 on the total MAGIC score To evaluate the rate of durable overall response rate from Day 28 to Day 56 To evaluate duration of response from Day 56 on the total MAGIC score To assess the individual durations of lower GI response (according to the MAGIC score) To assess the individual durations of lower GI response (according to the MAGIC score) in subjects that were re-treated with apraglutide because of a lower GI-aGVHD flare To assess the time to partial lower GI-aGVHD response as defined by the MAGIC score To assess the time to complete lower GI-aGVHD response as defined by the MAGIC score To assess best overall response Assessment of failure-free survival (FFS[1]) To assess NRM[2]

[1] The time from the date of randomization/Day 0 to the date of hematologic disease relapse/progression, non-relapse mortality, or addition of new systemic aGVHD treatment

[2] The time from date of randomization/Day 0 to the date of death not preceded by hematologic disease relapse/progression

	To assess overall survival (OS[3]) To assess the incidence of malignancy relapse[4] To assess failure of the transplantation (graft failure[5]) To assess cumulative SS and RUX doses used To assess the incidence of infections and sepsis To assess the effect of the two dose ranges on safety, tolerability, and efficacy <u>Exploratory Objectives</u> To assess pharmacokinetics (PK) of apraglutide in subjects with SR lower GI-aGVHD who are taking SS and RUX with a population PK approach To assess changes from baseline in subject Quality of Life questionnaires To assess changes from baseline in global assessments questionnaires To assess changes from baseline in nutrition status (body weight and parenteral support) To assess changes from baseline in GI regeneration and barrier function To assess cumulative need for blood transfusions per subject To explore biomarker expression changes from baseline related to GI-aGVHD To assess the constitution of the stool microbiome from baseline To assess exposure–response relationships for selected safety and secondary endpoints To assess health economics figures (time to

[3] The time from the date of randomization/Day 0 to the date of death due to any cause

[4] The time from date of randomization/Day 0 to hematologic disease relapse/progression

[5] <u>Primary graft failure:</u>

Absolute neutrophil count <0.5 × 10^9/L by Day 28
Hemoglobin <80 g/L and platelets <20 × 10^9/L
Reduced intensity conditioning: Confirmation of donor cell origin is required
Cord blood transplant: Up to Day 42

<u>Secondary graft failure:</u>

Absolute neutrophil count <0.5 × 10^9/L after initial engraftment not related to relapse, infection, or drug toxicity
Reduced intensity conditioning: Loss of donor hematopoiesis to <5%

	discharge from hospital, number of readmissions to inpatient settings, and duration of readmissions)
Trial endpoints:	<u>Safety Endpoints</u> Adverse events (AEs; System Organ Class [SOC], frequency, and severity) Incidence of AEs of special interest (AESIs): o Injection site reactions o Gastrointestinal obstructions o Gallbladder, biliary, and pancreatic disease o Fluid overload o Colorectal polyps o New malignancies o Systemic hypersensitivity Occurrence of clinically significant changes from baseline in clinical chemistry (including liver function tests), hematology, hemostasis, and urinalysis Occurrence of clinically significant changes from baseline in vital signs (blood pressure, heart rate) Occurrence of clinically significant changes from baseline in electrocardiogram (ECG) measurements (intervals and rhythm) Occurrence and titer of anti-drug antibodies (ADAs) <u>Secondary Endpoints</u> Overall response rate (PR and CR) at Day 56 on the lower GI tract MAGIC score Overall response rate (PR and CR) at Days 14, 28, 91, 119, 147, and 182 on the lower GI tract MAGIC score Overall response rate (PR and CR) at Days 14, 28, 56, 91, 119, 147, and 182 by organ system (skin, lower and upper GI tract, and liver) on the total MAGIC score Proportion of all subjects who achieve a CR or PR at Day 28 and maintain a CR or PR at Day 56 Duration of response from Day 56 (median and range) on the total MAGIC score where duration of response is defined as the interval from the Day 56 response (PR and CR) to death or new systemic therapy for aGVHD (including an increase in steroids

	>2 mg/kg/day methylprednisolone [MP] equivalent), whichever occurs first, with at least 182 days of follow-up Duration of response from Day 28 (median and range) on the total MAGIC score where duration of response is defined as the interval from the Day 28 response (PR and CR) to death or new systemic therapy for aGVHD (including an increase in steroids >2 mg/kg/day MP equivalent), whichever occurs first, with at least 182 days of follow-up Individual duration of lower GI response (according to the MAGIC score) counted from the first response to return to baseline or worse Individual duration of lower GI response (according to the MAGIC score) in subjects that were re-treated with apraglutide because of a lower GI-aGVHD flare, counted from the first response after apraglutide restart to return to baseline or worse Time to partial lower GI-aGVHD response (median and range) as defined by the MAGIC score Time to complete lower GI-aGVHD response (median and range) as defined by the MAGIC score Best overall response defined as overall response (PR or CR) at any time point up to and including Day 91 and before the start of additional systemic therapy for lower GI-aGVHD Failure-free survival up to 2 years post-first dose of apraglutide Non-relapse mortality up to 2 years post-first dose of apraglutide Incidence of malignancy relapse up to 2 years post-first dose of apraglutide Overall survival up to 2 years post-first dose of apraglutide Incidence of graft failure up to 2 years post-first dose of apraglutide Incidence of lower GI-aGVHD flare up to Day 182 after the first apraglutide dose following earlier cessation due to complete lower GI-aGVHD response Cumulative SS and RUX doses from start of the RUX treatment up to Day 91 after the first dose of apraglutide

	Incidence of infections and sepsis from baseline to Day 91 after the first dose of apraglutide Effect of the two dose groups on safety/tolerability and efficacy <u>Exploratory Endpoints</u> Pharmacokinetics of apraglutide assessed with a population PK approach. Absorption rate constant (ka), apparent clearance (CL/F), and apparent volume of distribution (Vz/F) with their intra- and inter-individual variability derived using a nonlinear mixed effects modeling approach, from sparse samples Quality of life and changes in subject-reported outcomes from baseline (EuroQol-5 dimensions-5 levels [EQ-5D-5L], Functional Assessment of Cancer Therapy – Bone Marrow Transplantation [FACT-BMT]) Global assessment questionnaires (Physician Global Assessment of Disease, Patient Global Assessment of Severity, and Patient Global Assessment of Change) Body weight and parenteral support (volume and caloric content) at Days 14, 28, 56, 91, 119, 147, and 182 compared with baseline Individual need for blood transfusions (cumulative bags per subject) assessed from baseline to Day 91 Biomarker expression, clinical chemistry parameters, and lower GI histology (when available) related to GI-aGVHD, GI regeneration, and GI barrier function as assessed at baseline and at times indicated in the schedule of assessments: o Citrulline (measure of intestinal repair and regeneration) in serum o Regenerating islet-derived protein 3 alpha (REG3α) in serum o Suppression of tumorigenicity 2 (ST2) in serum o MAGIC algorithm probability (MAP) score consisting of REG3α and ST2 o Angiopoetin-1 and -2 in serum o Soluble thrombomodulin (sTM) in serum o Vascular endothelial growth factor (VEGF) in serum

	○ Albumin in serum ○ Bilirubin in serum ○ Presence of intestinal cell lines (L-cells, Paneth cells, intestinal stem cells) and status of mucosal architecture (crypts, villi) from histology slides of lower GI biopsies before apraglutide treatment and at the Day 56 visit (when biopsy data are available) ○ Calprotectin in stool ○ Microbial constitution of the stool microbiome Time to discharge from hospital, number of readmissions to an inpatient setting and duration of readmissions up to Week 26
Trial design:	This is a randomized, single-blind, proof-of-concept trial to evaluate the safety, tolerability, and preliminary efficacy of apraglutide in subjects with Grade II to IV SR lower GI-aGVHD who are taking BAT. <u>Screening/Baseline</u> The screening process can start any time after clinical diagnosis of lower GI-aGVHD and SS initiation. The subject should sign the ICF before any other trial procedure. This period will last at maximum 12 weeks (Days -84 to Day -1). Subjects will be randomized (Day 0/Week 0) and receive the first dose of apraglutide only if they developed SR lower GI-aGVHD and have started treatment with RUX Thirty subjects, weighing ≥50.0 kg, will be randomized to two treatment arms (high dose or low dose of apraglutide) based on their body weight at baseline (three body weight ranges: 50.0<60.0 kg, 60.0≤80.0 kg, and >80.0 kg). In addition to the 30 subjects, a separate, non-randomized cohort of up to four subjects with body weights from 40.0 to <50.0 kg will be assigned to receive 2.5 mg of apraglutide (low body weight [LBW] dose). <u>Treatment</u> Up to 34 subjects will receive apraglutide on a background of RUX and SS. Apraglutide will be administered subcutaneously once weekly for 8 weeks (Week 0 to Week 7, inclusive). Treatment can be continued up to 13 weeks (Week 8 to Week 12, inclusive) if no complete lower GI-aGVHD response is achieved at Week 8. <u>Optional Treatment</u>

	Optional treatment for additional 13 weeks (Week 13 to Week 25, inclusive) is allowed if no complete lower GI-aGVHD response is achieved at Week 13. Optional treatment can only be started if additional apraglutide treatment would benefit the subject based on the Investigator's judgement. Lower GI-aGVHD Flare Treatment Lower GI-aGVHD flare treatment can start as per the protocol if a subject develops a lower GI-aGVHD flare between Weeks 9 and 25, and if treatment with apraglutide was previously stopped when lower GI-aGVHD CR was achieved at Day 56 (or when PR or CR was achieved at Day 91). Only one event of a lower GI-aGVHD flare can be treated with apraglutide until no later than Week 25. Follow-up Follow-up will start at the following occasions: At Week 8 (if a complete lower GI-aGVHD response is achieved at Week 8). Visits at Weeks 8, 13, 17, 21, 26, 52, and 104/end of trial (EOT) should be performed At Week 13 (if complete lower GI-aGVHD response is achieved at Week 13). Visits at Weeks 13, 17, 21, 26, 52, and 104/EOT should be performed Once CR is achieved during the optional treatment or lower GI-aGVHD flare treatment. The visits to be performed will depend on when the subject achieve a lower GI-aGVHD CR After early treatment discontinuation, the subject will perform an early treatment discontinuation visit approximately 4 weeks (+1 week) from the last apraglutide dose and subsequently transition to follow-up visits at Weeks 26, 52, and 104/EOT The follow-up period will last up to 2 years from the first apraglutide dose. Early Treatment Discontinuation In case of early treatment discontinuation, the subject will be asked to return for an early treatment discontinuation visit approximately 4 weeks (+1 week) from the last apraglutide administration. The subject will then transition to follow-up visits at Weeks 26, 52, and 104/EOT. Early Trial Discontinuation In case of early trial discontinuation, the subject will be

	asked to return to the site approximately 4 weeks (+1 week) from the date of early trial discontinuation. All the assessments foreseen at the EOT visit will be performed. After this visit, there will be no further visits. Independent Safety Review Committee Following the randomization of the first subject, an independent Safety Review Committee (iSRC) will convene approximately every 4 weeks. The iSRC will review the data and provide independent safety and trial conduct oversight of the first six randomized subjects (all iSRC details such as the constitution, meeting frequency, and decision rules are detailed in the iSRC charter). Assuming no issues are identified during the ongoing iSRC reviews then 24 additional subjects, weighing ≥50.0 kg will be randomized to the two treatment arms. Randomization of these additional subjects can occur while iSRC monitoring is ongoing.
Inclusion/exclusion criteria:	Inclusion Criteria: 1. Signed informed consent for this trial prior to any trial specific assessment. A signed assent form will also be required for all subjects under the age of 18 years 2. Male or female subjects aged 12 years or above at the time of consent and who weigh a minimum of 40.0 kg. Only subjects aged 18 years and above will be included **in Germany and France** 3. Have undergone allogeneic hematopoietic stem cell transplantation (alloSCT) from any donor source (matched unrelated donor, sibling, haplo-identical) using bone marrow, peripheral blood stem cells, or cord blood. Recipients of non-myeloablative, myeloablative, and reduced intensity conditioning are eligible 4. Evident myeloid and platelet engraftment confirmed prior to trial medication start: a. Absolute neutrophil count >1000/mm^3 and b. Platelets ≥20,000/mm^3 Use of growth factor supplementation (granulocyte-colony stimulating factor and granulocyte-macrophage colony-stimulating factor) and transfusion support is allowed 5. Clinical diagnosis of lower GI-aGVHD, **MAGIC**

	Stage 1–4 prior to randomization. Suitable diagnostic procedures should be implemented to exclude alternative reasons for diarrhea; these include (but not limited to) fecal cultures and lower gut biopsy with histological assessment for infectious diseases 6. Clinically confirmed SR lower GI-aGVHD defined as subjects administered SS, given alone, or combined with calcineurin inhibitors (CNIs) and either: a. Disease progression based on organ assessment after 3 days of systemic MP ≥2 mg/kg/day[6] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) ±CNIs or b. Did not improve after 7 days of treatment with systemic MP ≥2 mg/kg/day[6] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) or c. Progressed to a new organ after treatment with systemic MP ≥2 mg/kg/day[6] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) for skin and upper GI-aGVHD or d. Recurred during or after a steroid taper. Initial dose should be ≥2 mg/kg/day[6] systemic MP ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) 7. Treatment with SS plus RUX (RUX started concomitantly to apraglutide or a maximum of 72 hours before apraglutide initiation) 8. Women of childbearing potential must agree to use a highly effective method of contraception and refrain from donating eggs during the trial and for 4 weeks after the EOT visit. Effective contraceptive 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

[6] The SS dose should be calculated based on the subject's actual weight, within the maximum lower margin of 15% from the calculated dose.

	tubal occlusion; vasectomized partner. **In Germany**, oral methods of hormonal contraception are to be combined with another accepted method of contraception. 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, may be confirmed with follicle-stimulating hormone test in case of doubt). 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 lactational amenorrhea method 9. Male subjects with a female partner of childbearing potential must commit to practice methods of contraception and abstain from sperm donation during the trial and for 2 weeks after the EOT visit. Nevertheless, if their partners are women of childbearing potential, they must agree to practice contraception and 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 <u>Exclusion Criteria:</u> 1. Treatment with any systemic GVHD therapy (other than SS and RUX) including methotrexate and mycophenolate mofetil at the time of randomization/Day 0. Graft versus host disease prophylaxis (ciclosporin A, tacrolimus, sirolimus, everolimus, or anti-thymocyte globulin) is allowed 2. Concomitant treatment with Janus kinase inhibitor therapy other than RUX at the time of randomization 3. Failed alloSCT due to relapse of underlying

	malignant disease 4. Presence of SR GI-aGVHD occurring after donor lymphocyte infusion for pre-emptive treatment of malignancy recurrence 5. Ongoing participation in an interventional trial or administration of any investigational drug in less than its five half-lives prior to randomization/Day 0. Participation in observational or interventional trials involving supportive care such as probiotics or antiemetics, graft manipulation or transplant procedures, new combinations or new dosing of approved therapies for conditioning, prophylaxis[7], pre- or post-alloSCT and treatment of the underlying malignant disease are allowed after consultation with the Sponsor 6. Known or suspected hypersensitivity to GLP-1 or GLP-2 analogs or apraglutide excipients 7. Any use of enteral glutamine or growth factors such as native GLP-2, GLP-1, or GLP-2 and GLP-1 analogs, or known ADA, within 6 months prior to randomization/Day 0 8. Inability to understand or unwillingness to adhere to the trial visit schedules and other protocol requirements including those subjects not willing to comply owing to drug or alcohol abuse or any condition that would, in the Investigator's judgment, interfere with full participation in the trial, including administration of trial medication and attending required trial visits, pose a significant risk to the subject, or interfere with interpretation of trial data 9. Less than 2 weeks anticipated survival at screening 10. Evidence of chronic renal disease as demonstrated by inadequate renal function, which is defined as estimated glomerular filtration rate (eGFR) <20 mL/min/1.73 m^2 (using the Chronic Kidney Disease Epidemiology [CKD-EPI] formula) and is confirmed within 48 hours before randomization/ Day 0 11. Presence of decompensated liver cirrhosis Child Pugh Classes B and C 12. Clinically significant or uncontrolled cardiac disease including acute myocardial infarction within 6 months

[7] Except methotrexate and mycophenolate mofetil

	prior to randomization/Day 0 uncontrolled hypertension, congestive heart failure New York Heart Association Class III or IV 13. Requirement for vasopressor or inotropic support within 30 days prior to randomization/Day 0 14. Presence of uncontrolled cholestatic disorders or unresolved sinusoidal obstructive syndrome/veno-occlusive disease of the liver (defined as persistent bilirubin abnormalities not attributable to aGVHD and ongoing organ dysfunction) 15. Presence of relapsed primary malignancy or treatment for relapse after alloSCT 16. Requirement for unplanned immune suppression withdrawal as treatment of early malignancy relapse or low donor chimerism. Unclear remission states will be discussed with the Sponsor 17. Presence of newly diagnosed malignancies at screening or prior to randomization/Day 0 18. History of chronic gall bladder or bile duct inflammation or biliary obstruction, unless a cholecystectomy was performed before screening 19. Presence or history of GI tumors (including the hepatobiliary system and pancreas) within the last five years before randomization; presence of colonic polyps that are not removed 20. Subjects that present or have a history of familial adenomatous polyposis 21. Presence of an active clinically uncontrolled infection or evidence of active tuberculosis (clinical diagnosis per local practice). Cytomegalovirus reactivation is permitted as long as no evidence of pulmonary disease is present 22. Central venous catheter sepsis requiring systemic antibiotics within the previous 7 days prior to randomization/Day 0 23. Known cGVHD 24. Known active GI inflammation not related to GI-aGVHD (e.g., active inflammatory bowel disease such as Crohn's and ulcerative colitis) 25. History of progressive multifocal leuko-encephalopathy 26. Known pregnant or nursing (lactating) women

	27. Known major abdominal surgery in the last 6 months prior to randomization/Day 0 (surgical feeding tube placement or other minimally invasive surgery is allowed) 28. History of clinically significant intestinal adhesions increasing the risk of GI obstruction or GI contrast examination findings suggesting subacute intestinal obstruction or stricture within 6 months prior to randomization/Day 0 29. Liver enzymes meeting any of the following criteria within 48 hours prior to trial medication start: a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >8 x upper limit of normal (ULN) b. Alanine aminotransferase or AST >5 x ULN AND international normalized ratio >3
Background therapy	Apraglutide will be given as an add-on-therapy to RUX and SS. Systemic steroid dose adjustments are allowed at the Investigator's discretion, and topical steroids may be added as needed. Ruxolitinib should be administered as per national guidelines (including dose adjustments). Apraglutide should be administered concomitantly to RUX. In exceptional cases where concomitant administration is not possible, RUX should be administered at maximum 72 hours before apraglutide initiation. Should a worsening of a subject's lower GI-aGVHD symptoms occur early after starting apraglutide, it is recommended to adjust SS/RUX, but to stay on the same CNI (dose and type) used before baseline.
Allowed/prohibited medications	<u>Allowed Medications</u> Concomitant medications/therapies for prophylaxis (e.g., ciclosporin A, tacrolimus, sirolimus, everolimus, or anti-thymocyte globulin) and treatment of SR lower GI-aGVHD, except the drugs/classes mentioned as prohibited Treatment with SS, RUX, and CNIs. These treatments should be tapered according to institutional guidelines based on the Investigator's judgement. However, it is recommended to stay on the same CNI (dose and type) used before baseline Supportive care, pain control drugs, antibiotics, antiemetics, and transfusions

	Growth factor supplementation (granulocyte-colony stimulating factor and granulocyte-macrophage-colony stimulating factor), donor lymphocyte infusions, and transfusion support <u>Prohibited Medications</u> Treatment with any systemic GVHD therapy other than SS and RUX including methotrexate and mycophenolate mofetil at the time of randomization/Day 0 and during the trial Concomitant treatment with Janus kinase inhibitor therapy (except RUX) at time of randomization/Day 0 and during the trial Citrulline supplements, any use of growth hormone, enteral glutamine or growth factors such as native GLP2, GLP1, or GLP-2 and GLP-1 analogs other than the investigational medicinal product under investigation within 6 months before randomization/Day 0 and during the trial Any investigational drug with the exception of the investigational drugs allowed as per exclusion criterion number 5
Trial product/intervention:	Apraglutide 2.5 mg, 4.0 mg, 5.0 mg, 7.5 mg, or 10.0 mg subcutaneous (SC) once weekly
Control intervention:	Historical comparison
Trial duration:	Up to 116 weeks. After the screening period (up to 12 weeks), subjects will be involved in the trial for approximately 24 months (8 or 13 weeks of treatment period and a follow-up period lasting up to 2 years after the first dose)
Principal Investigator:	Prof. Robert Zeiser, Universitaetsklinikum Freiburg, Germany
Trial centers:	Approximately 30 sites in Europe and North America
Statistical considerations:	<u>General</u> The principal statistical goal of this trial is to estimate the likelihood of efficacy of apraglutide as an add-on to BAT in treating subjects with SR lower GI-aGVHD after alloSCT. As such, the statistical analysis will not focus upon p-value generation but rather estimation and quantification of uncertainty. It is planned to randomize 30 subjects on a 1:1 basis to either a low or high dose of apraglutide. The efficacy analyses will be performed using Bayesian

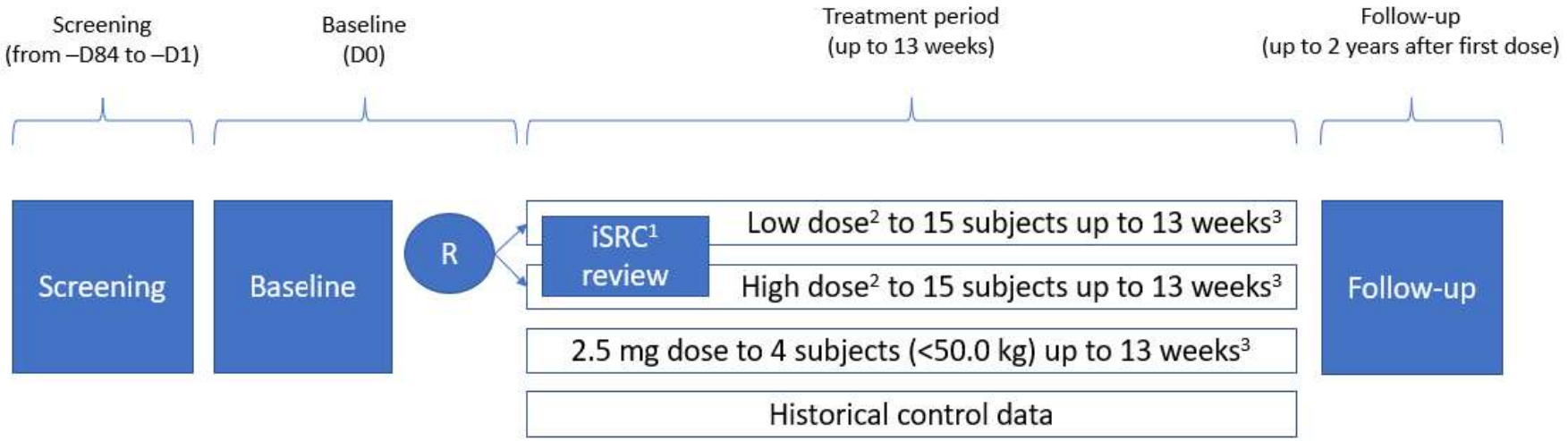
	methods comparing the observed results in each treatment arm with a historical control dataset. Analysis An interim analysis will be performed when 17 subjects have either reached Day 56 or withdrawn from the trial. All available data points by the data cut-off date will be included in the analysis. The final analysis will be done in two steps. The primary analysis of safety and efficacy will be performed once all subjects have either completed Day 91 or withdrawn from the trial. Additional analyses will be done once all the subjects have either completed the EOT visit or withdrawn from the trial. A Clinical Trial Report (CTR) will be issued after the primary analysis. An addendum to the CTR will cover the follow-up period until 2 years after the first apraglutide dose. Sample Size Up to 34 subjects with SR lower GI-aGVHD who are taking BAT will be randomized in this trial. The sample size justification was based on a selected secondary endpoint (Day 56 GI response). Observed response rates of 5/7 (71%) subjects at the interim analyses will lead to a high probability (>88%) of effectiveness comparing apraglutide and BAT with a historical Day 56 response rate derived from the previous trial, REACH2, including a 10% penalty. Analysis Sets The Full Analysis Set (FAS) comprises all enrolled subjects (randomized and non-randomized LBWs). All efficacy evaluations will be conducted using the FAS according to the planned dose (high dose, low dose, LBW dose) and in total. The LBW Analysis Set comprises all subjects with a body weight ranging from 40.0 to <50.0 kg assigned to receive 2.5 mg apraglutide who actually received at least one dose of therapy and who have provided at least one post-baseline assessment for any endpoint. All endpoints will be analyzed for this set separately. Safety Analysis Set 1 (SAS1) comprises all randomized subjects exposed to either of the two randomized apraglutide dose levels. All principal safety analyses will be carried out on the SAS1 according to the dose of apraglutide received. Safety Analysis Set 2 (SAS2) comprises all randomized and non-randomized subjects exposed to any apraglutide

	dose. Supportive safety analyses will be carried out on the SAS2 according to the dose of apraglutide received. Analysis of Secondary Endpoints Day 56 lower GI-aGVHD responses will be summarized and analyzed formally using Bayesian methods. In the Bayesian analyses, the response rates will be modelled by Beta distributions presenting the mean of the Beta distributions-associated with 90% credible intervals. The differences in response rates will be taken as the difference in mean response rates of the distributions on apraglutide with respect to BAT. The associated 90% credible interval will be computed using simulations of the respective distributions. Secondary endpoints measured as response/non-response will be analyzed in the same fashion as described above. If prior data for BAT is not available for a given time point, then only the apraglutide data will be presented. Secondary endpoints measured as time-to-event will be summarized using Kaplan-Meier analysis. Where there are competing risks, time-to-event data will also be summarized using cumulative incidence. The median time-to-event will be presented where possible, along with the associated 90% confidence interval. For comparison to BAT where prior data exist, the distribution of time-to-event will be modelled via the inverse gamma distribution, and thus compared on a Bayesian basis. Secondary endpoints will be summarized descriptively in the LBW Analysis Set. Exploratory Endpoints These endpoints will also be summarized descriptively in both the FAS and the LBW Analysis Sets. Analysis of Safety Endpoints All safety data will be summarized by apraglutide dose level for both SAS1 and SAS2. Adverse Events All AESIs, treatment-emergent AEs (TEAEs), serious AEs, AEs leading to discontinuation, AEs leading to death, and laboratory abnormalities will be summarized by apraglutide dose level, severity, and relationship to treatment. Adverse event data will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and presented by SOC and Preferred Term (PT).

Good Clinical Practice statement:	This trial will be conducted in compliance with the protocol, current version of the World Medical Association Declaration of Helsinki, the recent International Council on Harmonisation – Good Clinical Practice guidelines and all applicable national and local regulations.

TRIAL SCHEDULE

Figure 1: Trial Design



aGVHD=acute graft versus host disease; CR=complete response (of lower GI-aGvHD); D=Day; GI=gastrointestinal; iSRC=Independent Safety Review Committee; R=randomization

1. Review of the first six subjects randomized to two treatment arms
2. Exact dose depends on the weight of a subject a baseline (randomization/Day 0)
3. Apraglutide will be administered weekly for 8 weeks (Week 0 to Week 7, inclusive). Treatment can be continued up to 13 weeks (Week 8 to Week 12, inclusive) if a CR is not achieved at Week 8. If there is no CR at Week 13 optional treatment is allowed for a maximum of 13 weeks (Week 13 to Week 25, inclusive) or until a complete lower GI-aGVHD response is achieved, whichever comes first. Optional treatment can only be started if additional apraglutide treatment would benefit the subject based on the Investigator's assessment. Treatment can be re-started in case of a lower GI-aGVHD flare during follow-up (between Week 9 and Week 25) at the same dose given at randomization/Day 0 providing that apraglutide treatment was previously stopped if a lower GI-aGVHD CR was achieved at Day 56 (or a PR or CR was achieved at Day 91. Only one re-treatment course is allowed and should not last more than 13 weeks

Table 1: Schedule of Assessments - Screening, Baseline, Treatment, and Optional Treatment

Assessments	Screening[1]	Baseline /First dose	Treatment visits Week 1 – Week 7[2]							Treatment visits Week 8 – Week 12 *In case of no CR at Week 8*[3]	Optional Treatment visits[4] *If no CR at Week 13*						
											Lower GI-aGVHD flare treatment after CR is achieved[5]						
Visit No. *IRT/eCRF use*	**1**	**2**	**3**	**4**	**5**	**6**	**Dosing**	**7**	**Dosing**	**8**	**Dosing**	**9**	**Dosing**	**10**	**Dosing**	**11**	**Dosing**
Week		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9, 10, 11, 12**	**13**	**14, 15, 16**	**17**	**18, 19, 20**	**21**	**22, 23, 24, 25**
Day		**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**		**91**		**119**		**147**	
Visit Window[6]	-84 to -1 days	0	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
General																	
Informed consent	X																
Inclusion and exclusion criteria	X	X															
RUX start[7]		X															
Randomization[8]		X															
Demographics	X																
Medical, surgical and disease history	X																
Prior and concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hospitalization status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety																	
Colonoscopy/CT colonography/ MR enterography[9]	X																
Lower GI biopsy[10]	X									X							
Physical examination	X	X	X	X	X	X		X		X		X		X		X	
Twelve-lead ECG[11]	X	X															
Vital signs[12]	X	X	X	X	X	X		X		X		X		X		X	
Body weight and height[13]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy test[14]	X	X				X				X		X		X		X	
Local labs[15]	X	X[16]	X	X	X	X	Bilirubin only	X	Bilirubin only	X	Bilirubin only	X	Bilirubin only	X	Bilirubin only	X	Bilirubin only
Viral load[17]		X															
Central Lab																	
Albumin, citrulline, calprotectin, microbiome		X[18]	X	X	X	X		X		X		X		X		X	
Blood biomarkers		X[18]	X	X	X	X		X		X		X		X		X	

Assessments	Screening[1]	Baseline /First dose	Treatment visits Week 1 – Week 7[2]							Treatment visits Week 8 – Week 12 *In case of no CR at Week 8*[3]		Optional Treatment visits[4] *If no CR at Week 13*					
											Lower GI-aGVHD flare treatment after CR is achieved[5]							
Visit No. *IRT/eCRF use*	**1**	**2**	**3**	**4**	**5**	**6**	**Dosing**	**7**	**Dosing**	**8**	**Dosing**	**9**	**Dosing**	**10**	**Dosing**	**11**	**Dosing**
Week		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9, 10, 11, 12**	**13**	**14, 15, 16**	**17**	**18, 19, 20**	**21**	**22, 23, 24, 25**
Day		**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**		**91**		**119**		**147**	
Visit Window[6]	-84 to -1 days	0	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day	±1 day
Blood samples for pre-dose PK [19]			X	X	X	X		X		X							
Blood samples for post-dose PK (6, 30, 72, 120, and 168 h)[20]		X				X											
Blood samples for ADA[21]		X[18]	X	X		X				X		X		X		X	
Stool sample collection[22]		X	X	X	X	X			X			X		X		X	
Other assessments																	
Survival and disease progression		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Parenteral support[23]		X	X	X	X	X		X		X		X		X		X	
MAGIC assessment[24]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaires[25]		X		X		X		X		X		X		X		X	
Trial medication																	
Subcutaneous injection of IMP[26]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

ADA=anti-drug antibody; aGVHD=acute graft versus host disease; CR=complete response; CT=computed tomography; ECG=electrocardiogram; GI=gastrointestinal; IMP=investigational medicinal product; MAGIC= Mount Sinai Acute Graft Versus Host Disease International Consortium; MR=magnetic resonance; PK=pharmacokinetics; RUX=ruxolitinib;

Footnotes to Table 1:

1. The screening process can start any time after clinical diagnosis of lower GI-aGVHD and SS initiation; obtaining informed consent should be done before any other trial procedure:
   - If needed, screening can be performed on the same day as baseline, as long as all assessments required at screening and baseline are performed and the results are available **prior to randomization**
   - If the same assessment is foreseen at screening and baseline, and screening and randomization occur on the same day, then the assessment does not need to be repeated
2. Treatment should be continued until Week 7 inclusive, even if a complete lower GI-aGVHD response is achieved before Week 7 inclusive
3. If a lower GI-aGVHD CR is not achieved at Week 8, treatment should be continued until Week 12 inclusive; even if a CR is achieved before Week 12
4. Optional treatment can start if there is no complete lower GI-aGVHD response at Week 13 and if the Investigator considers that the subject could benefit from the additional apraglutide treatment. Treatment can continue for a maximum 13 weeks (until Week 25, inclusive) or until a complete lower GI-aGVHD response is achieved, whichever comes first
5. In case of lower GI-aGVHD flare, treatment can be re-started at the same dose given at randomization/Day 0 (e.g., if flare occurs at Week 17, treatment will be performed during visits at Weeks 17–25 or until a CR is achieved, whichever comes first). Treatment can continue for a maximum of 13 weeks (until Week 25, inclusive) or until a complete lower GI-aGVHD response is achieved, whichever comes first
6. All visit procedures are recommended to be done on the same day, unless specified otherwise
7. Ruxolitinib treatment should be initiated concomitantly with apraglutide (in exceptional cases where concomitant administration is not possible, RUX should be administered at maximum 72 hours before apraglutide initiation)
8. Randomization will be performed via IRT with subjects assigned to receive a low dose or high dose of apraglutide based on the weight band they fall into at baseline. Subjects below 50.0 kg will not be randomized but assigned into an open-label treatment group and will receive apraglutide 2.5 mg
9. **In Germany**, only colonoscopies or MR enterography can be performed to detect polyps (not CT colonography). At screening, historical data are allowed where the colonoscopy/CT colonography/ MR enterography was performed within 6 months prior to the screening visit
10. The screening and Day 56 lower GI biopsy samples are optional and should be obtained only if the subject is medically suitable for the procedure. If lower GI biopsy was not obtained at screening, the Day 56 lower GI biopsy is not required. Historical lower GI biopsies collected at clinical diagnosis of lower GI-aGVHD are allowed to be used to confirm eligibility (if a biopsy is needed to rule out other reasons for diarrhea) and further trial analysis
11. If screening and randomization occur on the same day, historical ECG results obtained within 48 hours can be used. If randomization is done within 48 hours from screening, ECG does not need to be repeated
12. Vital signs (blood pressure, heart rate, temperature) will be recorded before apraglutide administration at each treatment visit
13. Height is to be measured at screening only
14. For females of childbearing potential, serum pregnancy tests will be performed at screening and baseline. At all other visits, only a urine pregnancy test will be performed
15. Hematology, hemostasis, clinical chemistry, and urinalysis. At dosing visits (Weeks 5, 7, 9–12, 14–16, 18–20, 22–25), only bilirubin is collected
16. Samples can be taken within 48 hours prior to randomization. Creatinine clearance must be estimated (the estimated globular filtration rate) within 48 hours prior to randomization to determine subject eligibility. At all the other visits, only serum creatinine will be assessed
   - If screening and randomization occur on the same day, historical local lab results obtained within 48 hours can be used
   - If randomization occurs within 48 hours from screening, local lab results do not need to be repeated
17. For subjects with a positive historical serology result post-transplantation for Epstein-Barr virus, cytomegalovirus, severe acute respiratory syndrome coronavirus 2 (SARS-COV-2), human immunodeficiency virus (HIV), hepatitis B virus, or hepatitis C virus. A sample must be collected within 7 days prior to randomization/Day 0
18. This sample can be taken within 48 hours prior to randomization
19. Pre-dose PK samples should be taken within 2 hours before IMP administration is administered at Weeks 1, 2, 3, 4, 6, and 8
   - If the dose is not given, the sample should be collected at any time of the visit and the actual time should be documented
   - In cases where apraglutide was not administered during the previous week, the pre-dose PK sample at the current visit can be skipped

20. At Week 0 and Week 4 (or if not possible at Week 4, then perform PK sampling at Week 5 or Week 6 or Week 7 or Week 8), samples are to be collected at 6, 30, 72, 120, and 168 hours post-dose. Samples are to be taken within ±2 hours window, except for the 6-hour post-dose sample where only ±1 hour window is permitted. The actual time of PK sample collection must be documented. The 168-hour post-dose sample is not to be collected if a pre-dose sample is collected for the subsequent IMP administration
21. Anti-drug antibody samples must be collected prior to dosing. In addition, unscheduled samples should be collected in the case of an adverse event is judged as relevant by the Investigator (e.g., hypersensitivity)
22. Stool sample collection can be done within 24 hours prior to the visit (further instructions in the laboratory manual)
23. Defined as the average daily volume and caloric content of parenteral support used in the previous 7 days
24. If RUX and apraglutide do not start on the same day, MAGIC assessment should be done at screening and at RUX initiation. Historical data is allowed, if available, if RUX was started before the subject consented
25. The following questionnaires should be completed: EuroQol-5 Dimension – 5 Level Survey (EQ-5D-5L), Functional Assessment of Cancer Therapy – Bone Marrow Transplantation (FACT-BMT; will be completed only by the subjects ≥18 years old), Patient Global Assessments of Severity, Patient Global Assessment of Change (should not be completed at the baseline), and Physician Global Assessment of Disease. It is recommended that the questionnaires are completed at the beginning of the visit before other procedures are performed
26. Dosing must be performed on site. Subjects must be observed for 1 hour after each injection for potential adverse events. It is recommended to perform IMP administration in the morning to avoid performing PK sample collection during the night

Table 2: Schedule of Assessments - Follow-up

Assessments	Follow-up Visits NOTE: The number of follow-up visits will depend on when the subject will achieve CR during treatment[1]								
Visit No. *IRT/eCRF use*	**8**	**9**	**10**	**11**	**12**	**13**	**14/EOT**	**Early Treatment Discontinuation[2]**	**Early Trial discontinuation[3]**
Week	**8**	**13**	**17**	**21**	**26**	**52**	**104**	**NA**	**NA**
Day	**56**	**91**	**119**	**147**	**182**	**364**	**728**	**NA**	**NA**
Visit Window[4]	±1 day	±1 day	±1 day	±1 day	±1 day	±2 weeks	±4 weeks	4 weeks (+1 week) after last dose of apraglutide	4 weeks (+1 week) after date of trial discontinuation
General									
Prior and concomitant medication	X	X	X	X	X	X	X	X	X
Hospitalization status	X	X	X	X	X	X	X	X	X
Safety									
Colonoscopy/CT colonography/MR enterography[5]						X	X	X	
Lower GI biopsy[6]	X								
Physical examination	X	X	X	X	X	X	X	X	X
Twelve-lead ECG					X	X	X	X	X
Vital signs[7]	X	X	X	X	X	X	X	X	X
Body weight	X	X	X	X	X	X	X	X	X
Phone call[8]					Performed every 4 weeks				
Adverse events[9]	X	X	X	X	X	X	X	X	X
Pregnancy test	X	X	X	X	X	X	X	X	X
Local labs[10]	X	X	X	X	X	X	X	X	X
Central lab									
Albumin, citrulline, calprotectin, microbiome	X	X	X	X	X	X	X	X	X
Blood biomarkers	X	X	X	X	X	X	X	X	X
Blood samples for PK[11]	X								
Blood samples for ADA[12]	X	X	X	X		X	X	X	X
Stool sample collection[13]		X	X	X	X	X	X	X	X
Other assessments									
Survival and disease progression	X	X	X	X	X	X	X	X	X
Parenteral support[14]	X	X	X	X	X	X	X	X	X
MAGIC grade assessment	X	X	X	X	X	X	X	X	X
Questionnaires[15]	X	X	X	X	X	X	X	X	X

ADA=anti-drug antibody; CR=complete response; CT=computed tomography; ECG=electrocardiogram; eCRF=electronic Case Report Form; EOT=end of trial; GI=gastrointestinal; MAGIC= Mount Sinai Acute Graft Versus Host Disease International Consortium; MR=magnetic resonance; PK=pharmacokinetics; NA=Not applicable

Footnotes to Table 2:

1. Follow-up visits:
   - If a complete lower GI-aGVHD response is achieved by Week 8 inclusive, follow-up visits at Weeks 8, 13, 17, 21, 26, 52, and 104 should be completed
   - If a complete lower GI-aGVHD response is achieved from Week 9 to Week 13 inclusive, follow-up visits at Weeks 13, 17, 21, 26, 52, and 104 should be completed
   - If a complete lower GI-aGVHD response is achieved during the optional treatment period or lower GI-aGVHD flare treatment, perform the next follow-up visit available after a complete lower GI-aGVHD response is achieved (for example, if a complete lower GI-aGVHD response is achieved at Week 20, perform follow-up visits at Weeks 21, 26, 52, and 104)
2. In case of early **treatment** discontinuation, an early treatment discontinuation visit should be performed at approximately 4 weeks (+1 week) after the last IMP dose. Follow-up starts from the early treatment discontinuation visit and the subject will then transition to Weeks 26, 52, and 104 (EOT)
3. In case of early **trial** discontinuation, the subject will be asked to return to the site approximately 4 weeks (+1 week) from the date of early trial discontinuation. After this visit, there will be no further visits
4. All visit procedures are recommended to be done on the same day, unless otherwise specified
5. **In Germany**, only colonoscopy or MR enterography will be performed (not CT colonography)
6. The Day 56 lower GI biopsy is optional and should be done only if a lower GI biopsy was done prior to apraglutide therapy and if the subject is medically suitable for the procedure
7. Vital signs (blood pressure, heart rate, temperature)
8. A phone call must be performed every 4 weeks (±72 hours) between Weeks 26 and 104 to collect information regarding adverse events, concomitant medications, weight changes, and other pertinent information to assess the subject's medical condition. Phone calls will not be undertaken when the subject is attending the site for a scheduled visit
9. After Week 104/EOT visit, only SAEs that are judged as related to apraglutide will be reported. In case of early **trial** discontinuation, AEs should be collected for 4 weeks after the date of early trial discontinuation
10. Hematology, hemostasis, clinical chemistry, and urinalysis
11. If post-dose PK samples are collected after Week 7, the 168-hour (±2 hours) post-dose PK sample needs to be obtained. The actual time of PK sample collection must be documented
12. At Weeks 21, 52, and 104, samples will not be collected if treatment was stopped before the previous sample collection date and the previous sample result was negative (if results are available). Unscheduled samples should be collected in the case of an AE is judged as relevant by the Investigator (e.g., hypersensitivity)
    - Subjects who discontinue the trial prematurely will be asked to have ADA samples drawn at 4 (±2) and 8 (±2) weeks after an Early Trial Discontinuation visit. The Sponsor will inform the Principal Investigator of these results once available
13. Stool sample collection can be done within 24 hours prior to the visit (please refer to the laboratory manual)
14. Defined as the average daily volume and caloric content of parenteral support used in the previous 7 days
15. The following questionnaires should be completed: EuroQol-5 Dimension – 5 Level Survey (EQ-5D-5L), Functional Assessment of Cancer Therapy – Bone Marrow Transplantation (FACT-BMT; will be completed only for subjects ≥18 years old), Patient Global Assessments of Severity, Patient Global Assessment of Change, and Physician Global Assessment of Disease. The questionnaires are recommended to be completed at the beginning of the visit before other procedures are performed

1 INTRODUCTION

1.1 Background and Rationale

Allogeneic hematopoietic stem cell transplantation (alloSCT) is used to treat hematologic malignancies and other nonmalignant hematologic diseases and is a potentially curative treatment option. One of the major barriers to a successful transplant outcome is graft versus host disease (GVHD), which develops in 50–70% of patients who undergo alloSCT. Graft versus host disease is an inflammatory disorder which leads to tissue damage and ultimately organ failure and is categorized as either acute GVHD (aGVHD) or chronic GVHD (cGVHD).

Acute GVHD is most frequent within the first 100 days after transplantation and is the leading cause of non-relapse mortality (NRM) following alloSCT. Acute GVHD most typically affects the skin, gastrointestinal (GI) tract, and liver, and organ damage is characterized by apoptosis of cells.

Acute GVHD of the skin is generally not life-threatening, as both systemic and topical therapies are usually effective. While the incidence of Stage 3–4 liver GVHD is low (approximately 2%), the incidence of GI-aGVHD is reported to be between 40–50%. Treatment of GI-aGVHD remains unsuccessful in most cases, and the GI tract is involved in virtually all fatal cases of aGVHD.

Two distinct phenotypes of GI GVHD can be identified, upper GI (limited to Stage 1) and lower GI that differ in presentation, natural history, response to therapy, and risk of mortality. The upper GI phenotype generally does not progress to the lower GI phenotype. The upper GI phenotype presents with persistent loss of appetite, satiety, nausea, vomiting, and weight loss, with variable amounts of diarrhea, usually less than 500 mL per day. The presentation can be indolent, and therapy with prednisone at doses of 1 mg/kg/day plus topical oral corticosteroid is effective.

The lower GI phenotype of GVHD presents with secretory, protein-rich diarrhea and abdominal pain resulting from GI distention. In severe cases, the entire small intestine and colon are edematous and inflamed, with diarrheal volumes in excess of 1.5 L per day and evidence of mucosal ulceration and bleeding. Most patients with severe lower GI-aGVHD require prolonged hospitalization for supportive care including total parenteral support and pain control. Although outcomes are typically poor, the standard for initial therapy is prednisone, with the addition of other immune suppressive therapies when treatment with prednisone fails to control the disease.

Long-term survival rates in steroid refractory (SR) patients are poor and range between 5–30%.

Recently, ruxolitinib (RUX) was approved by the United States (US) Food and Drug Administration (FDA) for the treatment of SR-GVHD in 2019 based on the results of the REACH 1 trial. In this single arm trial, 54.9% (39 patients) had an overall response, including 26.8% (19 patients) with a complete response (CR); moreover, median duration of response was 345 days, and the overall survival estimate at 6 months was 51.0%. Despite this improvement in treatment options, there is still considerable unmet medical need in this population, as a significant number of patients either do not respond to RUX (30–40%) or lose the initial response over time.

Apraglutide is a potent glucagon-like peptide 2 (GLP-2) receptor agonist, specifically designed to have slower absorption, decreased clearance, increased resistance to

proteolysis, and increased plasma protein binding compared with the marketed GLP-2 analog, teduglutide. In addition, once weekly dosing with apraglutide, supported by pharmacokinetic (PK) data from previous trials, could substantially reduce the burden of therapy, improve subject compliance and adherence, and reduce adverse reactions associated with subcutaneous (SC) administration compared with teduglutide daily dosing.

When administered subcutaneously to healthy animals, apraglutide exhibited a trophic effect resulting in the increase of the weight of the small intestine (80–180% increase in Sprague-Dawley rats and 20–80% increase in minipigs across different dose levels and time points), and, to a lesser extent the large intestine as well [Apraglutide Investigator Brochure]. 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 the body weight loss and in 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 interfering with the anticancer efficacy of the chemotherapy, or with the efficacy of stem cell transplantation [Apraglutide Investigator Brochure].

In animals, apraglutide showed some beneficial effect on the bacterial flora (microbiota) through the protection of the intestinal mucosa allowing the maintenance of a normal equilibrium between the commensal bacterial populations [Norona, 2020]. In animal models of aGVHD, treatment with apraglutide improved animal survival and histopathological examination of intestinal segments showed less severe morphological effects through the entire intestine (including the colon) suggesting that this GLP-2 analog has a potential protective effect against the mucosal damage induced by the transplantation [Apraglutide Investigator Brochure].

The pharmacological properties of apraglutide suggest, therefore, that this peptide could have a therapeutic benefit in clinical conditions where the intestine is damaged by aggressive anticancer treatments or aGVHD. This view is further supported by evidence in humans with SR GVHD of the GI tract: a cohort of six subjects with SR aGVHD having failed multiple therapies were given teduglutide (another GLP-2 analog) at a dosage of 0.05 mg/kg body weight, once daily for 10 days. Intestinal biopsies taken from one subject before and during teduglutide treatment showed an increase in Paneth cells after the treatment. In addition to the increase in Paneth cells, clinical signs of intestinal GVHD improved in all six subjects with a decline in frequency of diarrhea. Serum albumin levels were analyzed in the six subjects and an increase in serum albumin was shown 2 weeks after treatment when compared with the day before treatment start. During the teduglutide treatment period no additional intravenous albumin or fresh frozen plasma was substituted. These findings suggest that GLP-2 may be effective in patients with SR GI-aGVHD, leading to an improvement in clinical symptoms, reduced loss of albumin, and regrowth of Paneth cells that secrete antimicrobial peptides, thereby improving the GI barrier function. This is in line with previous work showing that GLP-2 can increase nutrient absorption in normal rodents and in patients with short bowel syndrome (SBS).

Based on this background, apraglutide could open a new avenue of SR GI-aGVHD treatment by using a novel regenerative approach through GLP-2 signaling, a signaling cascade that may modulate the regeneration of lost enterocyte mass secondary to

inflammation by GVHD together with the repair of the impaired intestinal barrier function, resulting in improved intestinal absorption. These clinical features continue to be high unmet medical needs that contribute to the morbidity and mortality seen with acute SR GI-aGVHD.

The aim of this randomized, single-blind, proof-of-concept (POC) trial is to evaluate safety, tolerability, efficacy, durable overall response, and clinical outcomes of apraglutide in subjects with SR lower GI-aGVHD being treated with systemic steroids (SS) and RUX (current best available therapy [BAT]). The data will be compared with historical data from the RUX arm of the REACH2 trial when applicable. If available, alternative historical control cohorts will also be applied.

1.2 Investigational Medicinal Product and Indication

Apraglutide is a peptide analog of GLP-2 under development for treatment of SBS with intestinal failure (SBS-IF) and aGVHD, and it acts as a full agonist of 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 being resistant to cleavage by dipeptidyl peptidase-4, the major GLP-2 peptidase and increased plasma protein binding which prevents kidney elimination. The systemic half-life in various animal species, as well as in 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 the mucosal barrier function.

Apraglutide has been administered to 66 healthy volunteers and 16 subjects with SBS and has been proven to be safe and well tolerated at single doses ranging from 1.0 mg to 56.9 mg, and repeated weekly doses ranging from 1.0 mg to 28.4 mg. Further details are provided in the Investigator's Brochure. A Phase 3, placebo-controlled trial evaluating the efficacy and safety of apraglutide in subjects with SBS-IF is currently ongoing. At the time this protocol was written, 45 subjects have been recruited.

1.3 Non-clinical Evidence

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, and 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 [Hargrove, 2020].

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

1.4 Clinical Evidence

To date, four clinical trials with apraglutide have been completed, including the entry into human trial with a single ascending dose phase and a multiple ascending phase and one multiple dose Phase 1 trial, both in healthy volunteers and two Phase 2 trials designed to assess the safety and tolerability and to demonstrate the POC for apraglutide in subjects with SBS (5 mg and 10 mg). In these trials, apraglutide doses of up to 56.9 mg once weekly and up to 6 weeks duration (10 mg once weekly) have been tested.

In summary, apraglutide was safe and well tolerated after single and repeated doses (up to six weekly doses). In the entry in human Phase 1 trial, one subject showed increase in liver function test values at the highest dose (56.9 mg) in the multiple ascending dose part which resulted in the withdrawal of this subject. Nevertheless, overall, there were no relevant differences in adverse events (AEs) and tolerability across doses. Apraglutide was also safe and well tolerated with up to six weekly doses of 10 mg in a second Phase I healthy volunteer trial.

In the Phase 2 studies in SBS patients, the most frequent AEs included nausea, stoma output decreased, and stoma complications. Some AEs were related to the expected pharmacodynamic (PD) effects of apraglutide such as decreased stoma output, polyuria, and decreased thirst. Serious AEs (SAEs) reported in these studies were disease complications (device-related sepsis and device malfunction). Only one SAE of abdominal pain was assessed as related to apraglutide.

A summary of detailed observations from these studies is contained in the Investigator's Brochure [Apraglutide Investigator Brochure].

A Phase 3, placebo-controlled trial evaluating the efficacy and safety of apraglutide in subjects with SBS-IF is currently ongoing (Section 1.2). At the time this protocol was finalized 45 subjects had been recruited but no results are yet available.

1.5 Trial Objectives, Endpoints, and Rationale

1.5.1 Trial Objectives

1.5.1.1 *Primary Objective*

- To assess safety and tolerability of apraglutide in subjects with SR lower GI-aGVHD Grade II to IV Mount Sinai aGVHD International Consortium (MAGIC) who are treated with SS and RUX

1.5.1.2 *Secondary Objectives*

- To evaluate the overall response rate (partial response [PR] and CR) at Day 56 on the lower GI tract MAGIC score in subjects with SR lower GI-aGVHD Grade II to IV MAGIC that are treated with apraglutide, SS, and RUX compared to SS and RUX alone (BAT)
- To evaluate the overall response rate (PR and CR) at Days 14, 28, 91, 119, 147, and 182 on the lower GI-tract MAGIC score
- To evaluate the overall response rate (PR and CR) at Days 14, 28, 56, 91, 119, 147, and 182 on the total MAGIC score
- To evaluate the rate of durable overall response rate from Day 28 to Day 56
- To evaluate duration of response from Day 56 on the total MAGIC score
- To assess the individual durations of lower GI response (according to the MAGIC score)
- To assess the individual durations of lower GI response (according to the MAGIC score) in subjects that were re-treated with apraglutide because of a lower GI-aGVHD flare
- To assess the time to partial lower GI-aGVHD response as defined by the MAGIC score

- To assess the time to complete lower GI-aGVHD response as defined by the MAGIC score
- To assess best overall response
- Assessment of FFS[1]
- To assess NRM
- To assess overall survival (OS)
- To assess the incidence of malignancy relapse
- To assess failure of the transplantation (graft failure)
- To assess cumulative SS and RUX doses used
- To assess the incidence of infections and sepsis
- To assess the effect of the two dose ranges on safety, tolerability, and efficacy

1.5.1.3 *Exploratory Objectives*

- To assess PK of apraglutide in subjects with SR lower GI-aGVHD who are taking SS and RUX with a population PK approach
- To assess changes from baseline in subject Quality of Life questionnaires
- To assess changes from baseline in global assessments questionnaires
- To assess changes from baseline in nutrition status (body weight and parenteral support)
- To assess changes from baseline in GI regeneration and barrier function
- To assess cumulative need for blood transfusions per subject
- To explore biomarker expression changes from baseline related to GI-aGVHD
- To assess the constitution of the stool microbiome from baseline
- To assess exposure–response relationships for selected safety and secondary endpoints
- To assess health economics figures (time to discharge from hospital, number of readmissions to inpatient settings, and duration of readmissions)

1.5.2 Trial Endpoints

1.5.2.1 *Safety Endpoints*

- Adverse events (AEs; System Organ Class [SOC], frequency, and severity)
- Incidence of AEs of special interest (AESIs):
    - Injection site reactions
    - Gastrointestinal obstructions
    - Gallbladder, biliary, and pancreatic disease
    - Fluid overload
    - Colorectal polyps

[1] Definitions of these terms are provided in Appendix 16.2.

  - New malignancies
  - Systemic hypersensitivity
- Occurrence of clinically significant changes from baseline in clinical chemistry (including liver function tests), hematology, hemostasis, and urinalysis
- Occurrence of clinically significant changes from baseline in vital signs (blood pressure, heart rate)
- Occurrence of clinically significant changes from baseline in electrocardiogram (ECG) measurements (intervals and rhythm)
- Occurrence and titer of anti-drug antibodies (ADAs)

1.5.2.2 *Secondary Endpoints*

- Overall response rate (PR and CR) at Day 56 on the lower GI tract MAGIC score
- Overall response rate (PR and CR) at Days 14, 28, 91, 119, 147, and 182 on the lower GI tract MAGIC score
- Overall response rate (PR and CR) at Days 14, 28, 56, 91, 119, 147, and 182 by organ system (skin, lower and upper GI tract, and liver) on the total MAGIC score
- Proportion of all subjects who achieve a CR or PR at Day 28 and maintain a CR or PR at Day 56
- Duration of response from Day 56 (median and range) on the total MAGIC score where duration of response is defined as the interval from the Day 56 response (PR and CR) to death or new systemic therapy for aGVHD (including an increase in steroids >2 mg/kg/day methylprednisolone [MP] equivalent), whichever occurs first, with at least 182 days of follow-up
- Duration of response from Day 28 (median and range) on the total MAGIC score where duration of response is defined as the interval from the Day 28 response (PR and CR) to death or new systemic therapy for aGVHD (including an increase in steroids >2 mg/kg/day MP equivalent), whichever occurs first, with at least 182 days of follow-up
- Individual duration of lower GI response (according to the MAGIC score) counted from the first response to return to baseline or worse
- Individual duration of lower GI response (according to the MAGIC score) in subjects that were re-treated with apraglutide because of a lower GI-aGVHD flare, counted from the first response after apraglutide restart to return to baseline or worse
- Time to partial lower GI-aGVHD response (median and range) as defined by the MAGIC score
- Time to complete lower GI-aGVHD response (median and range) as defined by the MAGIC score
- Best overall response defined as overall response (PR or CR) at any time point up to and including Day 91 and before the start of additional systemic therapy for lower GI-aGVHD
- Failure-free survival up to 2 years post-first dose of apraglutide
- Non-relapse mortality up to 2 years post-first dose of apraglutide
- Incidence of malignancy relapse up to 2 years post-first dose of apraglutide
- Overall survival up to 2 years post-first dose of apraglutide

- Incidence of graft failure up to 2 years post-first dose of apraglutide
- Incidence of lower GI-aGVHD flare up to Day 182 after the first apraglutide dose following earlier cessation due to complete lower GI-aGVHD response
- Cumulative SS and RUX doses from start of the RUX treatment up to Day 91 after the first dose of apraglutide
- Incidence of infections and sepsis from baseline to Day 91 after the first dose of apraglutide
- Effect of the two dose groups on safety/tolerability and efficacy

1.5.2.3 *Exploratory Endpoints*

- Pharmacokinetics of apraglutide assessed with a population PK approach. Absorption rate constant (ka), apparent clearance (CL/F), and apparent volume of distribution (Vz/F) with their intra- and inter-individual variability derived using a nonlinear mixed effects modeling approach, from sparse samples
- Quality of life and changes in subject-reported outcomes from baseline (EuroQol-5 dimensions-5 levels [EQ-5D-5L], Functional Assessment of Cancer Therapy – Bone Marrow Transplantation [FACT-BMT])
- Global assessment questionnaires (Physician Global Assessment of Disease, Patient Global Assessment of Severity, and Patient Global Assessment of Change)
- Body weight and parenteral support (volume and caloric content) at Days 14, 28, 56, 91, 119, 147, and 182 compared with baseline
- Individual need for blood transfusions (cumulative bags per subject) assessed from baseline to Day 91
- Biomarker expression, clinical chemistry parameters, and lower GI histology (when available) related to GI-aGVHD, GI regeneration, and GI barrier function as assessed at baseline and at times indicated in the schedule of assessments:
    - Citrulline (measure of intestinal repair and regeneration) in serum
    - Regenerating islet-derived protein 3 alpha (REG3α) in serum
    - Suppression of tumorigenicity 2 (ST2) in serum
    - MAGIC algorithm probability (MAP[2]) score consisting of REG3α and ST2
    - Angiopoetin-1 and -2 in serum
    - Soluble thrombomodulin (sTM) in serum
    - Vascular endothelial growth factor (VEGF) in serum
    - Albumin in serum
    - Bilirubin in serum
    - Presence of intestinal cell lines (L-cells, Paneth cells, intestinal stem cells) and status of mucosal architecture (crypts, villi) from histology slides of lower GI biopsies before apraglutide treatment and at the Day 56 visit (when biopsy data are available)
    - Calprotectin in stool

[2] The MAP score is calculated using predicted probability (p) of 6-month NRM: $\log[-\log(1-p)] = -11.263 + 1.844 \log_{10}(ST2) + 0.577 \log_{10}(REG3\alpha)$

- Microbial constitution of the stool microbiome

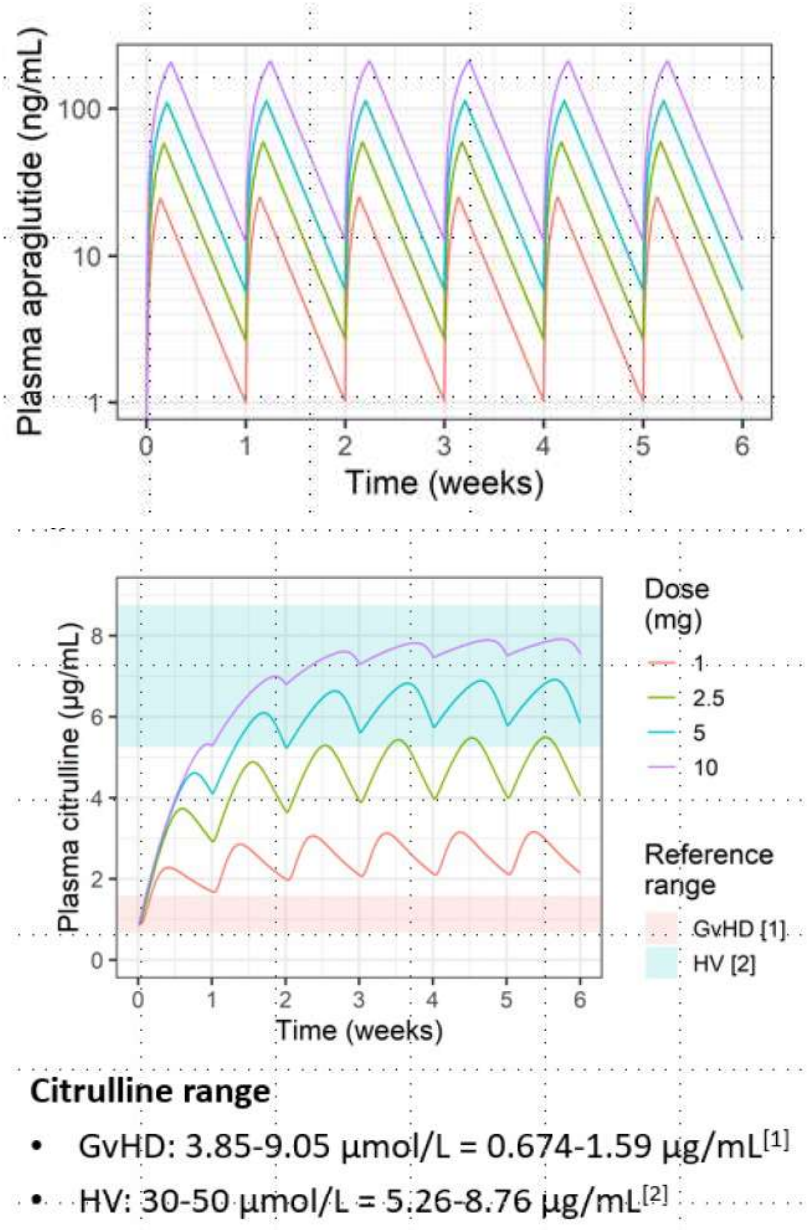
- Time to discharge from hospital, number of readmissions to an inpatient setting and duration of readmissions up to Week 26

1.6 Dose Rationale

It has been identified that plasma citrulline levels correlated with functional GI mucosal mass, and these levels were significantly lower in the plasma taken from patients with GI-aGVHD compared with healthy subjects as a result of the GI damage in the former [Vokurka, 2013].

To predict apraglutide doses required to return and maintain citrulline levels that are reduced to below 2 µg/mL in subjects with GVHD (red zone in Figure 2) back to normal levels of above 5 µg/mL (blue zone in Figure 2), the first approach used the PK/PD model developed on plasma apraglutide and plasma citrulline concentrations from 66 healthy volunteers and 16 subjects with SBS. Simulations of plasma apraglutide and plasma citrulline from the PK/PD model in Figure 2 show that apraglutide 5 mg and 10 mg dosed once weekly can restore and maintain citrulline in a range that is typical for healthy volunteers with an undisturbed GI tract.

Figure 2: Simulations of Plasma Apraglutide and Plasma Citrulline



GvHD=graft versus host disease; HV=healthy volunteers

1. Vokurka, 2013
2. Crenn, 2003

The exposure margins for 5 mg and 10 mg doses of apraglutide compared to those at the NOAELs in the Good Laboratory Practice (GLP) repeat-dose toxicity studies are well in excess of 200-fold in both rat and minipig studies relative to the exposure at the proposed clinical dose of apraglutide (5 mg and 10 mg once weekly via SC injection). The exposure margin for the proposed weekly clinical doses of 5 mg and 10 mg are >40-fold and >20-fold higher, respectively, than the NOAEL of 1 mg/kg/day in the 13-week GLP mouse toxicity trial. The exposure margins are 17- and 11-fold regarding the area under the curve (AUC) at steady state for the doses of 5 mg once weekly and

10 mg once weekly, respectively, at the NOAEL in the rabbit embryo fetal trial. Therefore, effective contraception will be mandatory during this clinical trial.

The safety of apraglutide doses used so far in clinical trials is summarized in Section 1.8.3.

The effective dose of apraglutide is not known in subjects with GI-aGVHD. Apraglutide has a broad therapeutic range and there is a large toxicity margin for the doses proposed in this trial. No differences were seen in the AE and SAE profile in doses up to 10 mg in both the healthy volunteer studies and to date in subjects with SBS. As this dose-finding trial aims to optimize GI stabilization and regeneration in a condition with a severely impaired organ system and a high morbidity and mortality rate, testing apraglutide at doses up to 10 mg once weekly is justified.

Nevertheless, as apraglutide has shown body weight dependency of AUC, the drug will be dosed according to four body weight categories in randomized subjects as summarized in Table 3. Subjects will be randomized to either a “high dose” or a “low dose” treatment group and will receive a dose according to their body weight range.

Table 3: Low and High Doses by Body Weight Bands and Respective Estimated Mean Exposure

		Dose		Estimated Mean AUC			
				Low		High	
		Low	High	Min	Max	Min	Max
Group	**Weight Range (kg)**	2.50		295	420		
1	**40–<50**						
2	**50–<60**	2.50	5.00	205	295	412	571
3	**60–<80**	4.00[1]	7.50	204	329	377	639
4	**80+**	5.00	10.00	121	257	237	497

AUC=area under the curve; Max=maximum; Min=minimum
NOTE: AUC range based on body weight 80–120 kg
1. For operational simplicity a 3.75-mg dose that was obtained in modelling was rounded up to 4.0 mg in the subjects weighing between 60 and 80 kg.

A separate, non-randomized cohort of up to four subjects with body weights ranging from 40.0–<50.0 kg will receive the low body weight (LBW) dose of apraglutide (2.5 mg).

1.7 Rationale of Trial Population Studied

The subject population selected for this trial has SR lower GI-aGVHD and is being treated with SS and RUX. Currently, this can be considered BAT for patients with this disease; however, a significant number of patients either do not respond to RUX (30–40%) or lose the initial response over time.

Amongst the three major organ systems involved in aGVHD (skin, liver, and GI), the GI is the key driver of morbidity and mortality in this disease. The addition of apraglutide as a treatment, complementary to the underlying immunosuppressive regimen, potentially acts via intestinal stabilization and regeneration and is expected to have a clinically relevant impact on this patient population with a high unmet medical need.

1.8 Risk/Benefit Considerations

1.8.1 Known Risks with Use of Glucagon-like Peptide Analogs

Teduglutide, the only approved and marketed GLP-2 analog, has shown good tolerability and safety with once daily administration [Kochar, 2018]. There are treatment-related risks reported that are related to the mode of action, such as possible risk of acceleration of neoplastic growth and enhancement of colon polyp growth [Revestive, SmPC].

Recently, a long-term safety update on teduglutide was presented [Allard, 2021] (ongoing prospective, observational, multinational SBS-IF registry; data cut-off June 30, 2020). This analysis compared long-term safety outcomes between adult subjects treated with teduglutide ("ever-treated") and those receiving standard of care and never exposed to teduglutide ("never-treated"). The per protocol analysis set included 467 ever-treated and 675 never-treated subjects; 306 subjects and 461 subjects, respectively, were included in the primary outcome analysis set. The authors reported incidences of benign polyposis that are well in line with the current knowledge on GLP-2. No malignancies were reported.

The most commonly reported adverse reactions in the teduglutide clinical trials were abdominal pain and distension (45%), respiratory tract infections (28%; including nasopharyngitis, influenza, upper respiratory tract infection, and lower respiratory tract infection), nausea (26%), infected site reactions (ISRs; 26%), headache (16%), and vomiting (14%). Approximately 38% of the treated subjects with a stoma experienced GI stoma complications. The majority of these reactions were mild or moderate [Revestive, SmPC]. Also, due to increased absorption there is a risk of fluid overload and of overdose with concomitant medications that have a narrow therapeutic window (Section 4.2.3.2).

The Sponsor has sufficient pre-clinical safety information to support the planned duration of treatment with apraglutide. This assessment is based upon the following: a completed (and reported in Investigational New Drug application 143'011) 26-week Good Laboratory Practice repeated dose toxicity trial with apraglutide in rats and a 39-week Good Laboratory Practice repeat-dose toxicity trial in minipigs that showed only the expected intestinotrophic pharmacological effect and no evidence of intestinal tumors or other adverse effects of apraglutide. Two-year carcinogenicity studies in rats and mice with apraglutide are ongoing. For further details, please refer to the Investigator's Brochure.

1.8.2 Assessment of Known and Potential Risks and Benefits

Several precautions are in place to increase the safety of participating subjects with SR lower GI-aGVHD in this trial:

- Subjects with a current or history of GI disease that might be at risk to develop GLP-2 typical adverse effects will be excluded from trial participation
- Subjects with known GI polyps will have their polyps removed before the start of the apraglutide treatment
- Mid- and longer-term follow-up investigations will be in place to check for newly emerging polyps
- A Sponsor-independent Safety Review Committee (iSRC) is in place to closely supervise subject safety during the trial and to make recommendations to the Sponsor with regards to the safe conduct of the trial

The overall assessment of the Sponsor's own safety data on apraglutide, as well as published data on the GLP-2 teduglutide and the anticipated benefit in the target population with high unmet medical need, concludes that the anticipated benefit of apraglutide treatment outweighs the individual risk for the participating subjects.

1.8.3 Summary of Clinical Safety with Apraglutide Reported During Phase 1 and Phase 2 Trials

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

- A dose relationship was not seen for any AE in any of the trials
- Doses up to 56.9 mg were safe and well tolerated in the single-ascending dose and multiple-ascending dose parts of the Phase 1 trial GYM-P3-698 in healthy volunteers
- Apraglutide 10 mg was well tolerated in healthy volunteers who received up to six weekly doses in trial TA799-002
- The most frequent AEs included nausea, GI stoma output decreased, and stoma complications
- Frequent AEs were primarily related to the expected efficacy effects of apraglutide, including decreased stoma output or stoma output abnormal, polyuria, and decreased thirst
- Serious AEs 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 assessed by the Investigator as related to apraglutide
- For each hematology, coagulation, and chemistry parameter, mean and median change 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 msec 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 msec 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 of Period 3
- Low-titer ADAs were seen in five subjects out of 64 treated (none in GYMP3698, one in TA799-002, three in GLY-311-2017, and one in GLY-311-2017). The ADAs had no apparent effect on either the PD or PK of apraglutide

In summary, the safety and tolerability profile of once weekly administration with apraglutide derived from clinical trials conducted so far confirms the expected mode of action-related safety effects. These should be anticipated to also occur with apraglutide in the current trial.

1.9 Rationale for Choice of Control

Published data from REACH2 was chosen to provide an approximate sample size estimation based on a selected secondary endpoint (Day 56 GI response).

During the trial, the Sponsor will pursue the option of establishing one or more alternative historical control cohorts better matched to the current trial population and the selected secondary endpoint.

2 TRIAL DESIGN

2.1 Rationale for the Trial Design

This POC trial is designed as a single-blind trial and aimed at assessing the safety and tolerability of three dose groups of apraglutide in subjects with SR lower GI-aGVHD receiving SS and RUX as underlying immunosuppressive therapy (BAT). Within the dose levels studied, subjects will receive an apraglutide dose according to their body weight at baseline.

This POC trial will also provide a preliminary assessment of the efficacy of apraglutide as a GI-regenerative approach on top of BAT in order to study a novel, complementary mode of action in a therapeutic setting that is historically dominated by immunosuppression.

Published randomized control trial data on BAT from REACH2 [Zeiser, 2020a] were initially chosen to derive a historical control.

As an increased use of RUX in combination with SS is used in standard of care, during the trial, the Sponsor will pursue the option of establishing one or more alternative historical control cohorts better matched to the current trial population and the selected secondary endpoint.

2.1.1 Rationale for the Independent Safety Review Committee

As this is the first time apraglutide will be administered to this population, intensive safety monitoring will occur. The iSRC will oversee the safety of trial participants and will make recommendations to the Sponsor as to the safe conduct of the trial. Further details are reported in Section 8. The iSRC charter describes the composition, objectives, structure, and responsibilities of the iSRC and the procedures the committee will follow.

2.2 Overall Design

This is a randomized, single-blind, POC trial to evaluate the safety, tolerability, and preliminary efficacy of apraglutide in subjects with SR lower GI-aGVHD Stage 1–4 who are taking BAT. The overall trial design is presented in Figure 1.

Screening will start any time after clinical diagnosis of lower GI-aGVHD and SS initiation, and the period will last a maximum of 12 weeks. Subjects will be randomized/dosed on Day 0 only if they develop SR lower GI-aGVHD and have started RUX. Overall, subjects will attend a screening visit, a baseline/first dosing visit, up to 12 treatment visits, up to an additional 13 treatment visits in case of optional or lower GI-aGVHD flare treatment, and up to seven follow-up visits depending on when the subject transitions from the treatment to follow-up period.

Thirty subjects will be randomized to receive a low or high dose of apraglutide. Subjects weighing ≥50.0 kg, will be randomized to two treatment arms (low or high dose of apraglutide) based on their body weight at baseline (three body weight ranges: 50.0<60.0 kg, 60.0≤80.0 kg, and >80.0 kg). In addition to the 30 subjects, a separate, non-randomized cohort of up to 4 subjects with body weights from 40.0 to <50.0 kg will be assigned to receive 2.5 mg of apraglutide (LBW dose).

Apraglutide will be administered subcutaneously once weekly from Week 0 to Week 7 inclusive.

In case of no complete lower GI-aGVHD response at Week 8, subjects will continue treatment from Week 8 to Week 12 inclusive.

Subjects with no complete lower GI-aGVHD response at Week 13 can optionally continue apraglutide for a maximum of 13 additional weeks (if the Investigator concludes that the subject is still benefitting from apraglutide treatment).

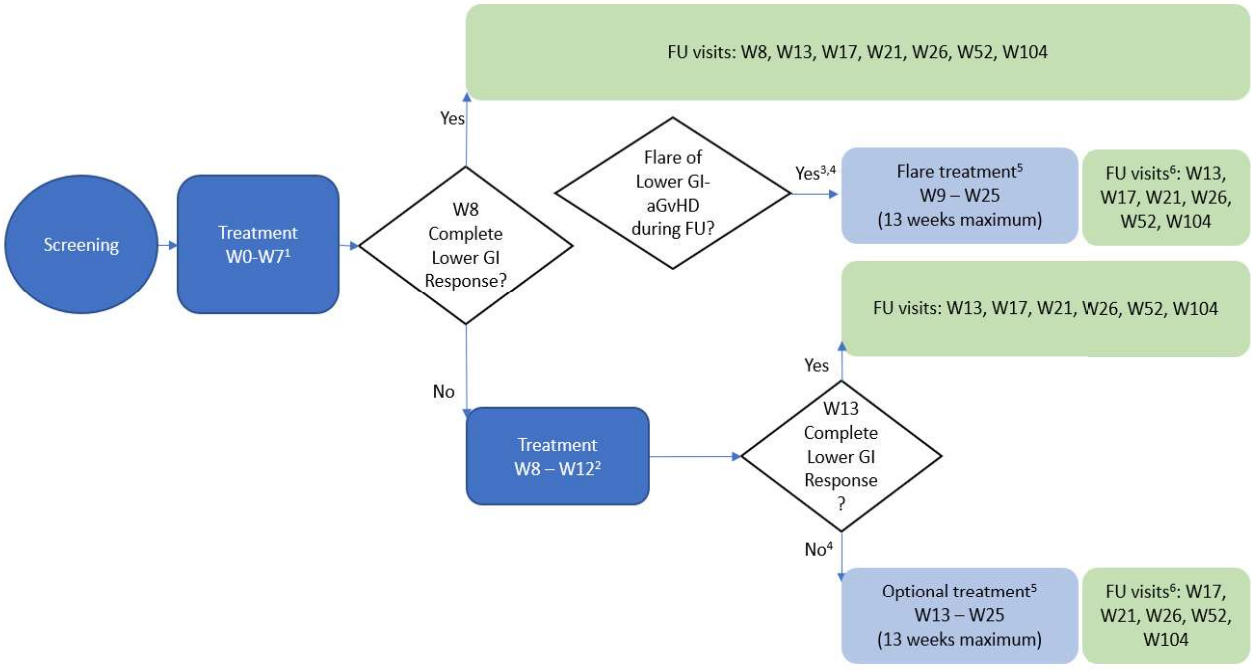
Treatment can be re-started in case of lower GI-aGVHD flare (e.g., if flare during Week 17, treatment will be performed once weekly between Week 17 and Week 25). Only one event of flare can be treated with apraglutide.

Follow-up will start at Week 8 (if complete lower GI-aGVHD response occurs by Week 8 inclusive), Week 13 (if complete lower GI-aGVHD response occurs between Week 9 and Week 13 inclusive), anytime once a complete lower GI-aGVHD response is achieved during the optional treatment or lower GI-aGVHD flare treatment, and after early treatment discontinuation. Follow-up will continue up to 2 years after the first dose of apraglutide.

Assessments scheduled during screening, baseline, treatment, lower GI-aGVHD flare and optional treatment periods are listed in Table 1. Assessments scheduled during the follow-up are presented in Table 2.

Treatment course and subject transition through the trial periods is presented in Figure 3.

Figure 3: Subject Flow Diagram



aGVHD=acute graft versus host disease; FU=follow-up; GI=gastrointestinal; GVHD=graft versus host disease; W=week

1. Subject to continue treatment up to Week 7 inclusive, even if complete lower GI-aGVHD response is achieved before Week 7 inclusive
2. Subject to continue treatment up to Week 12 inclusive, even if complete lower GI-aGVHD response is achieved between Week 8 and Week 12 inclusive
3. Lower GI-aGVHD flare treatment can be started after occurrence of a lower GI-aGVHD flare between Week 9 and Week 25 (e.g., if lower GI-aGVHD flare occurs during Week 17, treatment will be performed once weekly between Weeks 18–25). Only one episode of lower GI-aGVHD flare can be treated with apraglutide for a maximum of 13 weeks, no later than Day 176
4. Optional apraglutide treatment can be started if the Investigator concludes that the subject is still benefitting from apraglutide treatment at Week 13
5. Apraglutide dosing should be stopped once CR is achieved

6. Perform only the follow-up visits after complete lower GI-aGVHD response is achieved (e.g., if complete lower GI-aGVHD response was achieved at Week 20, perform follow-up visits at Weeks 21, 26, 52, and 104)

Following randomization of the first subject, an iSRC will convene approximately every 4 weeks. The iSRC will review the data and provide independent safety and trial conduct oversight of the first six randomized subjects. Assuming no issues are identified during the ongoing iSRC reviews then 24 additional subjects, weighing ≥50.0 kg, will be randomized to the two treatment arms. Randomization of these additional subjects can occur while iSRC monitoring is ongoing. The iSRC will continue the safety supervision of the trial.

An interim analysis will be performed when 17 subjects have either reached Day 56 or withdrawn from trial. All available data points by the data cut-off date will be included in the analysis.

The final analysis will be done in two steps. The primary analysis of safety and efficacy will be performed once all subjects have either completed Day 91 or withdrawn from the trial. Additional analyses will be done once all the subjects have either completed the EOT visit or withdrawn from the trial. A Clinical Trial Report (CTR) will be issued after the primary analysis. An addendum to the CTR will cover the follow-up period until 2 years after the first apraglutide dose.

2.2.1 Screening Period

Recruitment will be competitive across sites and countries.

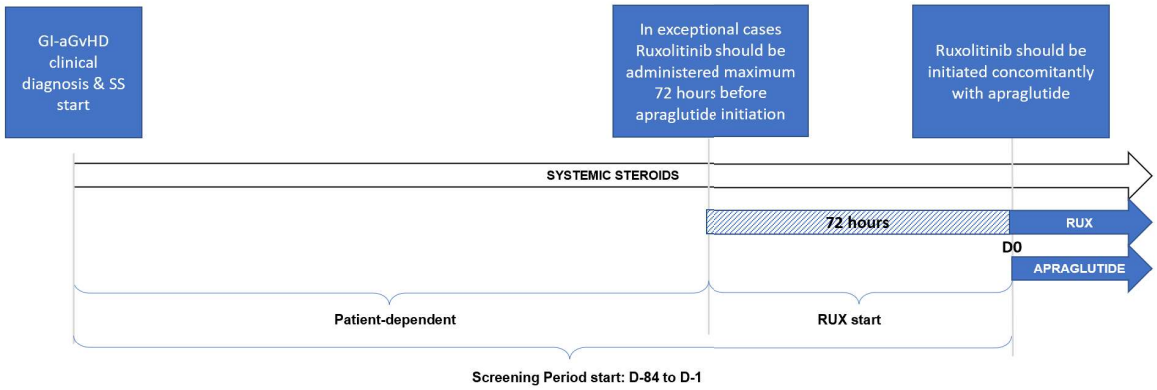
Only after obtaining written informed consent can protocol assessments start to determine subject eligibility (see Section 3.2). All eligibility criteria must be met for the subject to be allowed to be randomized at the Day 0 visit.

The screening process can start any time after lower GI-aGVHD diagnosis and SS initiation, and screening will continue for a maximum of 12 weeks until the subject becomes SR. Subjects will be considered eligible if they fulfil all eligibility criteria and after having developed SR lower GI-aGVHD started RUX.

Ruxolitinib should be administered as per national guidelines (including dose adjustments). Apraglutide should be administered concomitantly to RUX. In exceptional cases where concomitant administration is not possible, RUX should be administered at maximum 72 hours before apraglutide initiation.

A schematic graph of the screening period is illustrated in Figure 4.

Figure 4: Screening Period



aGvHD=acute graft versus host disease; D=day; GI=gastrointestinal; RUX=ruxolitinib

Screening activities may occur on more than one day after informed consent is signed. A colonoscopy is required to exclude presence of polyps at screening. A computed tomography (CT) colonography (where allowed) or magnetic resonance (MR) enterography can be used as an alternative to colonoscopy at the Investigator's discretion depending on the subject's medical condition. Historical data if obtained within 6 months prior to screening are allowed. A subject is eligible if no polyps are detected. In case polyps detected during screening, these should be removed prior to randomization/Day 0.

The screening period cannot be extended beyond 12 weeks as aGVHD may have transitioned into cGVHD, in which case the subject will be classified as a screen failure.

If needed, screening can be performed on the same day as baseline, as long as all assessments required at screening and baseline are performed and the results are available prior to randomization as per the Schedule of Assessments (Table 1).

If the same assessment is foreseen at screening and baseline, and screening and baseline are performed on the same day, then the assessment does not need to be repeated at baseline.

2.2.2 Treatment, Optional Treatment, and Lower GI-aGVHD flare Treatment

Eligible subjects, weighing ≥50.0 kg, will be randomized to one of the two treatment arms (low dose or high dose in one of the three weight bands). The exact dose they receive at each visit will depend upon the weight band they fall into at baseline (in the range from 2.5 mg to 10 mg, see Table 4). Further details are provided in Section 4.2.1.1. Subjects who weigh 40.0–<50.0 kg will not be randomized but assigned to receive apraglutide 2.5 mg SC once weekly (LBW dose).

Subjects will receive their first dose of apraglutide concomitantly with RUX. In exceptional cases where concomitant administration is not possible, RUX should be administered at maximum 72 hours before apraglutide initiation.

Apraglutide will be administered via SC injection weekly for either 8 or 13 weeks. Subjects will be dosed weekly from Week 0 to Week 7 inclusive.

Apraglutide should be given at least until Week 8 unless the Investigator sees intolerable AEs that are clearly attributable to apraglutide. This will ensure that apraglutide is given enough time to fully establish its GI stabilization and regeneration effects. Should a worsening of a subject's GI-related symptoms occur shortly after starting apraglutide, adjustment of SS/RUX is the first recommended step.

If there is no complete lower GI-aGVHD response at Week 8, subjects will continue treatment from Week 8 to Week 12 inclusive. Treatment between Weeks 0 and 7 or between Weeks 8 and 12 should be continued even if a complete lower GI-aGVHD response is achieved. If the Investigator concludes that a subject is still benefitting from apraglutide treatment and a subject has no complete lower GI-aGVHD response at Week 13, apraglutide can be optionally continued for a maximum of 13 additional weeks (until Week 25, inclusive) or until a complete lower GI-aGVHD response is achieved, whichever comes first.

Lower GI-aGVHD flare treatment can start as per the protocol if a subject develops a lower GI-aGVHD flare between Weeks 9 and 25, and if treatment with apraglutide was previously stopped when lower GI-aGVHD CR was achieved at Day 56 (or when PR or CR was achieved at Day 91). The definition of a lower GI-aGVHD flare is given in Appendix 16.2.

Only one event of a lower GI-aGVHD flare can be treated with apraglutide until no later than Week 25. Apraglutide can be restarted at the same dose received at randomization.

Treatment can continue for a maximum 13 weeks (until Week 25, inclusive) or until a complete lower GI-aGVHD response is achieved, whichever comes first.

If the lower GI-aGVHD flare continues after Week 25, standard aGVHD therapies should be introduced to control the lower GI-aGVHD flare.

If the lower GI-aGVHD flare is determined to be cGVHD with overlap syndrome, standard treatment of cGVHD with immunosuppressive therapy should be initiated.

2.2.3 Follow-up Period

The follow-up period will last up approximately 2 years from the first investigational medicinal product (IMP) administration and will start at different time points during the trial:

- If a complete lower GI-aGVHD response is achieved by Week 8 inclusive, the subject will transition to follow-up at Week 8. Follow-up visits at Weeks 8, 13, 17, 21, 26, 52, and 104/EOT should be performed
- If a complete lower GI-aGVHD response is achieved from Week 9 to Week 13 inclusive, the subject will transition to follow-up at Week 13. Follow-up visits at Weeks 13, 17, 21, 26, 52, and 104/EOT should be performed
- If a complete lower GI-aGVHD response is achieved during the optional treatment or lower GI-aGVHD flare treatment, the subject will transition to follow-up.E.g., if a complete lower GI-aGVHD response is achieved at Week 20, only the visits at Weeks 21, 26, 52, and 104/EOT should be performed
- In case of early treatment discontinuation, the subject will attend an early treatment discontinuation visit approximately 4 weeks (+1 week) from the last IMP dose and subsequently transition to follow-up visits at Weeks 26, 52, and 104/EOT

2.2.4 Phone Calls

Between Week 26 and Week 104/EOT, the site will contact the subject approximately every 4 weeks via telephone. Phone calls will not be made in the same week that a trial visit is scheduled. Unscheduled calls can be performed at the discretion of the Investigator.

2.2.5 Subject Treatment after End of Trial

A long-term extension trial is not planned.

2.2.6 Early Treatment Discontinuation

A subject who discontinues treatment early is NOT considered to be withdrawn from the trial, but rather the subject will be followed up until the planned Week 104/EOT visit, provided that the subject's consent has not been withdrawn.

An early treatment discontinuation visit should be scheduled approximately 4 weeks (+1 week) from the last IMP dose. The subject will then transition to follow-up visits at Weeks 26, 52, and 104/EOT. The same visit should be completed also in case of early treatment discontinuation during the optional treatment period.

The decision for early treatment discontinuation can be made by the subject, the Investigator, or Sponsor personnel. A subject has the right to prematurely discontinue

trial medication at any time, without any justification, by withdrawal from trial medication only or by withdrawal from any further participation in the trial (i.e., early trial discontinuation [see Section 2.2.7]).

Although a subject is not obliged to give his/her reason for prematurely withdrawing from the treatment or the trial, it is recommended that the Investigator makes a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

The Investigator must discontinue trial medication for a given subject if, on balance, he/she believes that continued administration would be contrary to the best interests of the subject.

A subject who discontinues treatment early and withdraws consent to participate in any further trial assessments is considered as withdrawn from the trial and no further assessment will be collected after informed consent withdrawal.

Apraglutide should be permanently stopped under the following conditions:

- New malignancy is identified during the trial (subjects must be treated and followed up as per local guidelines and practices for those malignancies)
- In case of an elevation of laboratory parameters as reported in Table 6
- The subject is pregnant
- In case of unacceptable toxicities associated (definitely, probably, possibly related) with apraglutide

For further considerations on IMP dose reduction and temporary discontinuation, see Section 6.6.2.

The Investigator must discontinue trial medication for a given subject if, on balance, he/she believes that continued administration would be contrary to the best interests of the subject.

A subject who discontinues treatment early and withdraws consent to participate in any further trial assessments is considered as withdrawn from the trial and no further assessment will be collected after informed consent withdrawal.

Guidance on stopping rules and follow-up medical care of subjects requiring dose adjustment are described in Section 4.2.1.2.

2.2.7 Early Trial Discontinuation

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 at least until the medical situation has resolved.

Regardless of the reason for withdrawal, the subject will be asked to return to the site approximately 4 weeks (+1 week) from the date of early trial discontinuation. After this visit, no further visits will occur.

In cases where further site visits are not possible, subjects should be asked if they would allow the Investigator to continue contacting them and reporting their safety data for 4 weeks after early trial discontinuation.

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

1. The subject chooses to withdraw

2. The Investigator judges it necessary due to non-compliance with trial procedures (including if the subject loses the ability to give consent)
3. The Investigator judges it necessary to protect the subject's best interest
4. 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
5. The subject is pregnant
6. The clinical trial is terminated by the Sponsor
7. The iSRC has recommended it

Subjects who discontinue the trial prematurely will be asked to have ADA samples drawn at 4 (±2) and 8 (±2) weeks after an Early Trial Discontinuation visit (refer to Table 2 and Section 5.9.2).

2.2.7.1 *Subjects "Lost to Follow-up" Prior to Last Scheduled Visit*

A subject is considered lost to follow-up when the Investigator cannot reach the subject. If contact details are available and permission has been obtained, the subject's relatives should be contacted by phone to obtain a reason for the subject not attending a visit (e.g., an AE). If at least three phone calls or other contact efforts (e.g., e-mails, text messages) at different times during a course of 3 weeks are not answered, a registered letter should be sent to the subject. All contact attempts should be documented in the subject's source documents. If the subject decides to withdraw early for the trial, the Investigator should encourage the subject to continue with follow-up safety assessments. If the subject returns to the site at a later time point, the Sponsor should be informed to discuss further steps.

2.2.7.2 *Replacement of Subjects*

Subjects that discontinue/withdraw after enrollment/randomization will not be replaced.

2.2.8 Screen Failures

The following data will be collected and entered into the electronic Case Report Form (eCRF) for all subjects that are screen failures:

- Date of informed consent form (ICF) and screening visit date
- Underlying disease history, GI-aGVHD history, history of transplant
- Demographics
- Eligibility criteria
- Screen failure reason and date
- Serious AEs, trial procedures-related AEs
- Concomitant medications/procedures related to AEs

If the subject does not meet the eligibility criteria, the subject will be considered a screen failure.

Subjects that failed screening may be rescreened once at a later stage if the reason for screen failure has changed over time (e.g., out of range laboratory values have resolved). Re-screening should occur at least 2 weeks after the time of screen failure and require (i) re-consenting and (ii) Sponsor approval. Subjects who are re-screened will receive a new subject number.

All screening assessments (except colonoscopy/CT colonography/MR enterography) will need to be repeated for the re-screened subject. **In Germany**, only colonoscopies or MR enterographies (not colonographies) can be performed.

2.3 End of Trial Definition

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

The Sponsor may terminate the trial early according to certain circumstances, for example, but not restricted to:

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

3 TRIAL POPULATION

3.1 Number of Subjects

This trial will include approximately 34 adult and pediatric subjects (12 years or older on the day of screening) who have SR lower GI-aGVHD. **In Germany and France**, only subjects 18 years old and above will be included in this trial.

Sufficient subjects will be screened to allow randomization of approximately 30 subjects to one of two treatment arms (low dose or high dose based on three body weight bands). Up to a further four subjects with a weight of 40.0–<50.0 kg will be treated with apraglutide 2.5 mg (LBW dose).

3.2 Eligibility Criteria

3.2.1 Inclusion Criteria

1. Signed informed consent for this trial prior to any trial specific assessment. A signed assent form will also be required for all subjects under the age of 18 years
2. Male or female subjects aged 12 years or above at the time of consent and who weigh a minimum of 40.0 kg. Only subjects aged 18 years and above will be included **in Germany and France**
3. Have undergone alloSCT from any donor source (matched unrelated donor, sibling, haplo-identical) using bone marrow, peripheral blood stem cells, or cord blood. Recipients of non-myeloablative, myeloablative, and reduced intensity conditioning are eligible
4. Evident myeloid and platelet engraftment confirmed prior to trial medication start:
   a. Absolute neutrophil count >1000/mm^3

      and

   b. Platelets ≥20,000/mm^3

   Use of growth factor supplementation (granulocyte-colony stimulating factor and granulocyte-macrophage-colony stimulating factor) and transfusion support is allowed
5. Clinical diagnosis of lower GI-aGVHD, **MAGIC Stage 1–4** prior to randomization**.** Suitable diagnostic procedures should be implemented to exclude alternative reasons for diarrhea; these include (but not limited to) fecal cultures and lower gut biopsy with histological assessment for infectious diseases
6. Clinically confirmed SR lower GI-aGVHD defined as subjects administered SS, given alone, or combined with CNIs and either:
   a. Disease progression based on organ assessment after 3 days of systemic MP ≥2 mg/kg/day[3] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) ±CNIs

[3] The SS dose should be calculated based on the subject's actual weight, within the maximum lower margin of 15% from the calculated dose

or

b. Did not improve after 7 days of treatment with systemic MP ≥2 mg/kg/day[3] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent)

or

c. Progressed to a new organ after treatment with systemic MP ≥2 mg/kg/day[3] ([or prednisone dose ≥2.5 mg/kg/day] or equivalent) for skin and upper GI-aGVHD

or

d. Recurred during or after a steroid taper. Initial dose should be ≥2 mg/kg/day[3] MP (or equivalent) systemic MP ([or prednisone dose ≥2.5 mg/kg/day] or equivalent)

7. Treatment with SS plus RUX (RUX started concomitantly to apraglutide or a maximum of 72 hours before apraglutide initiation)
8. Women of childbearing potential must agree to use a highly effective method of contraception and refrain from donating eggs during the trial and for 4 weeks after the EOT visit. Effective contraceptive 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. **In Germany**, oral methods of hormonal contraception are to be combined with another accepted method of contraception.

   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, may be confirmed with follicle-stimulating hormone test in case of doubt). 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 lactational amenorrhea method
9. Male subjects with a female partner of childbearing potential must commit to practice methods of contraception and abstain from sperm donation during the trial and for 2 weeks after the EOT visit. Nevertheless, if their partners are women of childbearing potential, they must agree to practice contraception and 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

3.2.2 Exclusion Criteria

1. Treatment with any systemic GVHD therapy other than SS and RUX including methotrexate and mycophenolate mofetil at the time of randomization/Day 0. Graft versus host disease prophylaxis (ciclosporin A, tacrolimus, sirolimus, everolimus, or anti-thymocyte globulin) is allowed
2. Concomitant treatment with Janus kinase inhibitor therapy other than RUX at the time of randomization
3. Failed alloSCT due to relapse of underlying malignant disease
4. Presence of SR GI-aGVHD occurring after donor lymphocyte infusion for pre-emptive treatment of malignancy recurrence
5. Ongoing participation in an interventional trial or administration of any investigational drug in less than its five half-lives prior to randomization/Day 0. Participation in observational or interventional trials involving supportive care such as probiotics or antiemetics, graft manipulation or transplant procedures, new combinations or new dosing of approved therapies for conditioning, prophylaxis[4], pre- or post-alloSCT and treatment of the underlying malignant disease are allowed after consultation with the Sponsor
6. Known or suspected hypersensitivity to GLP-1 or GLP-2 analogs or apraglutide excipients
7. Any use of enteral glutamine or growth factors such as native GLP-2, GLP-1, GLP-2 and GLP-1 analogs, or known ADA within 6 months prior to randomization/Day 0
8. Inability to understand or unwillingness to adhere to the trial visit schedules and other protocol requirements including those subjects not willing to comply owing to drug or alcohol abuse or any condition that would, in the Investigator's judgment, interfere with full participation in the trial, including administration of trial medication and attending required trial visits, pose a significant risk to the subject, or interfere with interpretation of trial data
9. Less than 2 weeks anticipated survival at screening
10. Evidence of chronic renal disease as demonstrated by inadequate renal function, which is defined as estimated glomerular filtration rate (eGFR) <20 mL/min/1.73 m^2 (using the Chronic Kidney Disease Epidemiology [CKD-EPI] formula) and is confirmed within 48 hours before randomization/Day 0
11. Presence of decompensated liver cirrhosis Child Pugh Classes B and C
12. Clinically significant or uncontrolled cardiac disease including acute myocardial infarction within 6 months prior to randomization/Day 0, uncontrolled hypertension, congestive heart failure New York Heart Association Class III or IV
13. Requirement for vasopressor or inotropic support within 30 days prior to randomization/Day 0
14. Presence of uncontrolled cholestatic disorders or unresolved sinusoidal obstructive syndrome/veno-occlusive disease of the liver (defined as

[4] Except methotrexate and mycophenolate mofetil

persistent bilirubin abnormalities not attributable to aGVHD and ongoing organ dysfunction)

15. Presence of relapsed primary malignancy or treatment for relapse after alloSCT
16. Requirement for unplanned immune suppression withdrawal as treatment of early malignancy relapse or low donor chimerism. Unclear remission states will be discussed with the Sponsor
17. Presence of newly diagnosed malignancies at screening or prior to randomization/Day 0
18. History of chronic gall bladder or bile duct inflammation or biliary obstruction unless a cholecystectomy was performed before screening
19. Presence or history of GI tumors (including the hepatobiliary system and pancreas) within the last five years before randomization; presence of colonic polyps that are not removed
20. Subjects that present or have a history of familial adenomatous polyposis
21. Presence of an active clinically uncontrolled infection or evidence of active tuberculosis (clinical diagnosis per local practice). Cytomegalovirus reactivation is permitted as long as no evidence of pulmonary disease is present.
22. Central venous catheter sepsis requiring systemic antibiotics within the previous 7 days prior to randomization/Day 0
23. Known cGVHD
24. Known active GI inflammation not related to GI-aGVHD (e.g., active inflammatory bowel disease such as Crohn's and ulcerative colitis)
25. History of progressive multifocal leuko-encephalopathy
26. Known pregnant or nursing (lactating) women
27. Known major abdominal surgery in the last 6 months prior to randomization/Day 0 (surgical feeding tube placement or other minimally invasive surgery is allowed)
28. History of clinically significant intestinal adhesions increasing the risk of GI obstruction or GI contrast examination findings suggesting subacute intestinal obstruction or stricture within 6 months prior to randomization/Day 0
29. Liver enzymes meeting any of the following criteria within 48 hours prior to trial medication start:
    a. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >8 x upper limit of normal (ULN)
    b. Alanine aminotransferase or AST >5 x ULN AND international normalized ratio >3

3.3 Lifestyle Considerations

Subjects will observe the contraception requirements laid out in inclusion criteria numbers 8 (female subjects) and 9 (male subjects).

Subjects should not use any drugs of abuse from screening until the EOT visit unless they have been prescribed (e.g., benzodiazepines).

4 TRIAL INTERVENTION/MEDICATION

4.1 Description of Investigational Medicinal Products

The IMP tested in this trial is apraglutide, a GLP-2 analog composed of 33 amino acids, containing natural and unnatural amino acids from non-animal origin. Apraglutide is manufactured by solid-phase peptide synthesis using 9-fluorenylmethyloxycarbonyl as an amine-protecting group. The formulation of apraglutide- is an aseptically manufactured freeze-dried powder for reconstitution with commercially available sterile water for injection. The IMP also contains the following excipients: glycine, L-histidine, mannitol, and sodium hydroxide. The pH of the reconstituted solution is approximately 8.3.

Apraglutide will be supplied in vials with 10 mg freeze-dried powder for reconstitution in sterile water prior to SC injection. Further details are provided in the Investigator's Brochure.

4.1.1 Packaging, Labeling, and Supply (Re-supply)

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

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

All dosing will occur at the trial sites. Details are included in the Pharmacy Manual.

4.1.2 Storage Conditions

The Investigator must ensure that the IMP 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 as described in the Pharmacy Manual and recorded in a temperature log as per the policies and guidelines of the site and prior to IMP dispensation. Any IMP experiencing temperature deviations outside the allowed range must be placed in quarantine (between 2–8°C) reported and evaluated prior to releasing it back into stock for use in the trial. Details are provided to the site in the Pharmacy Manual.

4.2 Administration of Experimental and Control Interventions

4.2.1 Experimental Intervention

4.2.1.1 *Administration of Apraglutide*

The dose to be administered to the subject at each time point depends on the treatment arm to which the subject is randomized and the subject's weight at baseline, as presented in Table 4. Non-randomized subjects with LBWs will only receive 2.5 mg of apraglutide.

Table 4: Dose of Investigational Medicinal Product Based on Treatment Arm and Weight

Treatment Arm	Weight Band (based on Baseline Weight)			
	40.0–<50.0 kg[1]	50.0–<60.0 kg	60.0–80.0 kg	>80.0 kg
Low dose (mg)	2.5	2.5	4	5
High dose (mg)		5	7.5	10

1. A non-randomized cohort of up to four subjects

The SC injection will be administered in the abdominal area or in the thigh. The injection site must be rotated such that an injection is administered at least 5 cm away from where the last injection was administered.

The IMP must be administered weekly as per scheduled (Table 1 and Table 2). Detailed instructions will be provided in the Pharmacy Manual for the site.

The Investigator is ultimately responsible for the appropriate preparation and administration of the IMP by assigning the appropriate site personnel and ensuring they are well-trained. The dose assigned through IRT will need to remain blinded to the subjects. Investigational medicinal product will not be administered to any person not enrolled in this trial.

4.2.1.2 *Apraglutide Dose Adjustments, Interruptions and Stopping Rules*

For subjects who do not tolerate the protocol dosing schedule, dose adjustments, or interruptions are mandated to allow the subjects to continue the trial medication and maintain apraglutide dosing for optimal treatment of aGVHD. The objective of these dose adjustment rules is to optimize response for each individual subject while avoiding clinically relevant toxicities attributed to apraglutide.

Dose reductions should be undertaken as outlined in Table 5.

Table 5: Dose Reduction Steps for Apraglutide

Original dose (mg)	2.5	4	5	7.5	10
New dose (mg)	0	2.5	4	5	7.5

Deviations to the recommended dose reductions, temporary interruptions, or permanent interruptions will need to be documented.

Subjects who are receiving multiple oncological treatments, or are dependent on parenteral support, develop abnormal liver function tests. In both cases bilirubin, ALT,

and/or AST may be elevated at baseline; therefore, the stopping rules for these subjects will use the increase from baseline instead of values from the ULN.

Table 6 depicts the dose adjustments for apraglutide dose in case of deviations of clinical safety parameters starting from Week 1.

Table 6: Apraglutide Dose Adjustments—Holding and Stopping Rules

Total bilirubin elevated (adapted from MAGIC) Use x ULN if normal at baseline (BSL) or x BSL if elevated at BSL	
>1.5–3.0 x ULN or BSL	**Recommendation:** Maintain dose level
>3.0–5.0 x ULN or BSL	**Mandatory:** reduce by 1 dose level Monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤3.0 x ULN/BSL: • If resolved in ≤14 days, then return to initial dose level • If resolved in >14 days, then maintain the dose level minus 1
>5.0–10.0 x ULN or BSL	**Mandatory: Hold dosing** Monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤3.0 × ULN/BSL: • If resolved in ≤14 days, then resume at initial dose level • If resolved in >14 days, then resume at dose level minus 1
>10.0 x ULN or BSL	**Mandatory: Hold dosing** Monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤3.0 × ULN/BSL: • If resolved in ≤14 days, then resume at dose level minus 1 • If resolved in >14 days, then STOP dosing The subject should be further monitored weekly (including LFTs), or more frequently if clinically indicated, until total bilirubin has resolved to baseline or stabilization over 4 weeks
AST or ALT elevated (normal BSL)	
>5.0–<8.0 x ULN for more than 2 weeks	**Mandatory: Hold dosing** Repeat LFTs as soon as possible, preferably within 48–72 hours from awareness of the abnormal results; monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤5.0 × ULN. Then: • If resolved in ≤14 days (which start after 2 weeks of elevation), then resume at initial dose level • If resolved in >14 days (which start after 2 weeks of elevation), **wait for resolution to ULN** then resume at dose level minus 1

≥8.0 –≤20.0 x ULN	**Mandatory: Hold dosing** Repeat LFTs as soon as possible, preferably within 48–72 hours from awareness of the abnormal results; monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤ULN Then resume at dose level minus 1
>20.0 x ULN	**Mandatory: STOP dosing**
AST or ALT elevated (elevated at BSL)	
>3.0 –<5.0 x BSL	**Mandatory: Hold dosing** Repeat LFTs as soon as possible, preferably within 48–72 hours from awareness of the abnormal results; monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤BSL. Then: • If resolved in ≤14 days, then resume same dose level • If resolved in >14 days, wait for resolution to BSL then resume at dose level minus 1
≥5.0–≤20.0 x BSL	**Mandatory: Hold dosing** Repeat LFTs as soon as possible, preferably within 48–72 hours from awareness of the abnormal results; monitor LFTs weekly, or more frequently if clinically indicated, until resolved to ≤BSL. Then resume at dose level minus 1.
>20.0 x BSL	**Mandatory: STOP dosing**
Combined AST/ALT and bilirubin elevation Use x ULN if normal at BSL or x BSL if elevated at BSL	
ALT/AST >3.0 × ULN/BSL and total bilirubin >2.0 × ULN/BSL	**Mandatory: Hold dosing** Assess causality of the investigational compound: • If relationship to apraglutide is suspected (definitely, probably, possibly related) **Stop dosing** • If another cause is identified manage with institutional guidance and restart treatment
Asymptomatic amylase and/or lipase elevation	
>1.0–1.5 x ULN	**Recommendation:** Maintain dose level
>1.5–2.0 x ULN	**Recommendation:** Maintain dose level
>2.0–5.0 x ULN	**Recommendation: Hold dosing** until resolved to Grade ≤2, then: • If resolved in ≤7 days, then resume at same dose level • If resolved in >7 days, then resume at dose level minus 1
>5.0 x ULN	**Recommendation: Stop dosing**
Pancreatitis	

Grade 2	**Recommendation:** Maintain dose level
Grade ≥3	**Mandatory: Stop dosing**
New onset of abdominal pain or worsening from BSL	
Grade ≥3	**Mandatory: Stop dosing**

ALT=alanine aminotransferase; AST=aspartate aminotransferase; BSL=baseline; LFT=liver function test; MAGIC=Mount Sinai Acute Graft Versus Host Disease International Consortium; ULN=upper limit of normal

Additional visits for unscheduled laboratory samplings or for safety reasons can be arranged as per the Investigator's judgment. In case of abnormal laboratory results of Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or higher assessed at a subject's local doctor, a re-assessment should be scheduled at the site.

4.2.2 Ruxolitinib and Steroid Medications

4.2.2.1 *Ruxolitinib Dose Adjustments*

Dose reductions and temporary interruptions of RUX treatment may be needed in SR lower GI-aGVHD subjects with thrombocytopenia, neutropenia, or elevated total bilirubin after standard supportive therapy, including growth-factors, anti-infective therapies, and transfusions. Dose adjustment recommendations for RUX should be followed according to the current regional recommendations (the European Union Prescribing Information [EUPI] and United States Prescribing Information [USPI]).

Dose adjustments of RUX should be documented in the source documentation and in the relevant eCRF page.

4.2.2.2 *Steroids Dose Adjustments*

The dose of SS administered may be changed at the Investigator's discretion based on the subject's aGVHD symptoms and clinical parameters such as white blood cell and thrombocyte counts; furthermore, topical steroids may be added at the discretion of the Investigator.

4.2.3 Prior and Concomitant Treatments

4.2.3.1 *Prior Medication/Therapy*

Prior treatment includes previous medications or interventions received in the past which are no longer ongoing at screening. Details of following medications **from the start of conditioning** are to be recorded in the source documents and entered in the eCRF:

- Any systemic aGVHD prophylaxis or therapy including methotrexate and mycophenolate mofetil, ciclosporin A, tacrolimus, sirolimus, everolimus, or anti-thymocyte globulin
- Janus kinase inhibitor therapy
- Any investigational drug including supportive care such as probiotics or antiemetics, graft manipulation or transplant procedures, new combinations or new dosing of approved therapies for conditioning, prophylaxis, pre- or post-alloSCT, and treatment of the underlying malignant disease

4.2.3.2 *Concomitant Medication/Therapy*

Concomitant treatment is any medication or therapeutic intervention ongoing at screening and any new medication received during the trial.

At every visit, the Investigator or a qualified designee will ask the subject about concomitant medication.

No clinical drug-drug interaction trials have been performed with apraglutide, but experimental settings revealed that apraglutide is not an inhibitor nor an inducer of the most common cytochromes tested, in line with apraglutide being a peptide. Experimental investigations revealed furthermore that apraglutide has only a minimal likelihood of causing clinically relevant transporter interactions (see the Investigator's Brochure for details).

Based upon the PD effect of apraglutide, there is a potential for increased enteral absorption of medicinal products. Subjects receiving oral concomitant medicinal products requiring titration or with a narrow therapeutic index (e.g., opioids, aminoglycosides, ciclosporin, carbamazepine, digoxin, digitoxin, flecainide, lithium, phenytoin, phenobarbital, rifampicin, theophylline, warfarin, or others) should be monitored closely, with therapeutic drug monitoring or other suitable clinical parameters at the discretion of the Investigator.

In case of pain, it is recommended that non-opioid therapies are tried and optimized before prescribing immediate-release opioids, rather than extended-release/long-acting opioids. It is also recommended to prescribe the lowest effective dosage, below 50.0 morphine milligram equivalent per day, and to evaluate benefits and harms with the subject within 1–4 weeks of starting opioid therapy or of dose escalation.

Subjects should be advised to consult the Investigator or designee prior to taking any prescribed or over-the-counter medications.

Concomitant treatments are to be recorded in the source documents and entered in the eCRF. In particular, the exact dose of SS and RUX taken throughout the trial must be clearly recorded in the eCRF including any dose adjustments.

Routine vaccinations (flu, tetanus, etc.) are allowed during the trial but must be entered into the eCRF. Vaccination for SARS-COV-2 is allowed but each vaccine administration and the type of vaccine must be entered into the eCRF. All AEs attributed to vaccination e.g., soreness at injection site, must be recorded in the eCRF.

4.2.3.3 *Prohibited Medication/Therapy*

- Treatment with any systemic GVHD therapy other than SS and RUX including methotrexate and mycophenolate mofetil at the time of randomization/Day 0 and during the trial
- Concomitant treatment with Janus kinase inhibitor therapy (except RUX) at time of randomization/Day 0 and during the trial
- Citrulline supplements, any use of growth hormone, enteral glutamine or growth factors such as native GLP2, GLP1, or GLP-2 and GLP-1 analogs other than the IMP under investigation within 6 months before randomization/Day 0 and during the trial
- Any investigational drug with the exception of the investigational drugs allowed as per exclusion criterion number 5

4.2.3.4 *Allowed Medication/Therapy*

- Concomitant medications/therapies for prophylaxis (e.g., ciclosporin A, tacrolimus, sirolimus, everolimus, or anti-thymocyte globulin) and treatment of SR lower GI-aGVHD, except the drugs/classes mentioned in Section 4.2.3.3
- Treatment with SS, RUX, and CNIs. These treatments should be tapered according to institutional guidelines based on the Investigator's judgement. However, it is recommended to stay on the same CNI (dose and type) used before baseline.
- Supportive care, pain control drugs, antibiotics, antiemetics, and transfusions
- Growth factor supplementation (granulocyte-colony stimulating factor and granulocyte-macrophage-colony stimulating factor), donor lymphocyte infusions, and transfusion support

4.3 Investigational Medicinal Product Handling

4.3.1 Reconstitution

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 (Table 7). 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.

Table 7: Reconstitution of Investigational Medicinal Product

	2.5 mg IMP	4 mg IMP	5.0 mg IMP	7.5 mg IMP	10.0 mg MP
Vial content (apraglutide)	10 mg	10 mg	10 mg	10 mg	10 mg
Number of vials used	1	1	1	2	2
Reconstitution with water for injection per vial	0.4 mL	0.4 mL	0.4 mL	0.4 mL	0.4 mL
Total volume administered	0.1 mL	0.16 mL	0.2 mL	0.3 mL	0.4 mL
Concentration of reconstituted solution	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL

IMP=investigational medicinal product

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 (0.1 mL for 2.5 mg, 0.16 mL for 4 mg, 0.2 mL for 5 mg, 0.3 mL

for 7.5 mg, and 0.4 mL for 10 mg) will be drawn from the vial into the syringe for SC injection. Two reconstituted vials are to be used to draw 0.3 mL and 0.4 mL for the 7.5 mg and 10 mg doses, respectively. After drawing up the syringe, it will be inspected for foreign particles and used if judged acceptable for administration.

4.3.2 Documentation and Compliance

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 and accountability log and, unless restricted by the site standard operating procedures (SOPs), will be verified by a monitor during the trial against used vials. Compliance will also be assessed during IMP accountability (see Section 4.3.3).

4.3.3 Investigational Medicinal Product Accountability

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 and interactive response technology (IRT) data. If the Monitor's verification against used vials is restricted by site SOPs, 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.

4.3.4 Return or Destruction of Investigational Medicinal Product

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 to the Clinical Trials Supplies vendor for destruction according to local site policy/procedures. If such final reconciliation by the Monitor is restricted by site SOPs, then this reconciliation will be performed in accordance with site-specific IMP destruction policies. Details are described in the Pharmacy Manual.

4.4 Randomization and Blinding

This trial is designed as single-blind trial (only subjects will be blinded regarding the apraglutide dose level received).

A single-blind trial design does not represent a bias in the interpretation of results given that the main efficacy measure is an objective measurement (i.e., MAGIC score) of the disease progression based on symptoms and laboratory results.

5 TRIAL SCHEDULE AND TRIAL ASSESSMENTS

The trial visits shall be conducted according to the Schedule of Assessments (Table 1 and Table 2). Subjects who are inpatients may be visited on the ward by site staff.

5.1 Screening/Baseline Assessments

5.1.1 Demography

Demographic and baseline characteristics include:

- Age and birth year
- Sex
- Race and ethnicity

These details will be recorded in the subject's medical chart and entered in the eCRF. Collection of this information will be highlighted in the ICF.

5.1.2 Medical, Surgical, and Disease History

Relevant medical and surgical history will be recorded in the eCRF only for eligible subjects (not for screen failures), as judged by the Investigator. The information collected for medical and surgical history must include:

- Diagnosis and baseline severity of ongoing diseases
- Date of onset
- Date of resolution (if not ongoing)

Additionally, at screening, any drug or alcohol abuse within the last 12 months prior to screening will be documented as medical history (MH).

The MH will be obtained by asking the subject and/or by inspecting his/her medical records.

For the documentation of the subject's SR lower GI-aGVHD type and disease history, the subject will be asked, and/or his/her medical records will be inspected for information on:

- Details of the initial malignancy
- Date of transplant and type of donor
- Conditioning scheme received
- Date of onset of aGVHD
- Symptoms of aGVHD
- Treatment to date for aGVHD and when SR developed

For documentation of eligibility the source documents should include details on the MH, prior treatment, imagistic or endoscopic assessments results as applicable, to sufficiently document for each criterion the eligibility of the subject.

5.2 Reporting of Prior and Concomitant Treatment

Prior and concomitant treatment as defined in Section 4.2.3 will be entered into the eCRF only for eligible subjects (not for screen failures). For screened subjects and screen failures prior and concomitant treatment will be collected in eCRF only if linked to an AE.

At every visit the Investigator or a qualified designee will ask the subject about concomitant medication.

Blood transfusions should be recorded as a concomitant procedure in the eCRF.

The subject should be advised to consult the Investigator or designee before taking any prescribed or over-the-counter medications. Any new medication use needs to be documented with the reason stated.

5.3 Quality of Life Questionnaires

Quality of life questionnaires will be completed at the times outlined in the Schedule of Assessments (Table 1 and Table 2). They are recommended to be completed at the beginning of the visit prior to other procedures being performed.

5.3.1 EuroQol-5 Dimension – 5 Level Survey

The EQ-5D-5L is a descriptive system that combines five dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) and the EuroQol visual analog scale (VAS) to characterize the participant's health on the day [EuroQol Group, 1990; Brooks, 1996; Rabin, 2001; Herdman, 2011]. A copy of this questionnaire is presented in Appendix 16.1.1.

Each dimension has five levels or response options, with Level 1 representing best health, and Level 5 representing worst health. The single digit for each dimension is then combined to create a five-digit value that describes the participant's health today. The EuroQol VAS assesses "how good or bad" the participant's health is today using a vertical VAS ranging from 0 (worst health you can imagine) to 100 (best health you can imagine). Participants choose a location on the vertical line, and the number associated with that location is recorded as their rating of health today.

5.3.2 Functional Assessment of Cancer Therapy – Bone Marrow Transplantation

The FACT-BMT is a 50-item questionnaire covering five areas: physical well-being, social/family well-being, emotional well-being, functional well-being, and additional concerns. Each item is marked on a five-point Likert scale (not at all, a little bit, somewhat, quite a bit, very much) in relation to the previous 7 days. A copy of this questionnaire is presented in Appendix 16.1.2.

The FACT-BMT will only be completed by subjects aged ≥18 years old.

5.4 Global Assessments

The Global Assessments will be completed at the times outlined in Table 1 and Table 2. They are recommended to be completed at the beginning of the visit prior to other procedures being performed.

5.4.1 Patient Global Assessment of Severity

The Patient Global Assessment of Severity is a single-item questionnaire using a seven-point verbal response scale to assess the current status of the subject's condition. Response options range from 0=absent to 4=extremely severe (Appendix 16.1.3).

5.4.2 Patient Global Assessment of Change

The Patient Global Assessment of Change is a single-item questionnaire using a seven-point verbal response scale, to assess overall change in the subject's status

since taking the IMP. Response options range from 2=a lot better to -2=a lot worse (Appendix 16.1.4).

5.4.3 Physician Global Assessment of Disease

The Physician Global Assessment of Disease is a single-item questionnaire using a five-point verbal response scale to assess the current status of the subject's condition. Response options range from 0=no sign of disease to 4=extremely severe (Appendix 16.1.5).

5.5 MAGIC Grade Assessment, Survival, and Disease Progression

The Investigator or designee will assess the subject's aGVHD using the MAGIC grading scale (Section 1.5.2.3 and Appendix 16.3) at the times outlined in the Schedule of Assessments (Table 1 and Table 2).

If RUX and apraglutide do not start on the same day, MAGIC assessment should be done at screening and at RUX initiation. Historical data is allowed, if available, if RUX was started before the subject consented.

The four target organs (skin, liver, upper GI, and lower GI) will be assigned a Stage (between 0 and 4) and the four Stages combined to assign the subject's disease as being Grade 0–IV.

Stool volume is converted in the equivalent number of episodes in the MAGIC score grading scale (i.e., on average 200 mL of diarrhea corresponds to one episode in adults). Formed or mostly-formed stools should not be quantified or counted towards the diarrhea volume. If the subject has diarrhea, the Investigator should assess whether the diarrhea is due to GVHD or to other confounding effects such as infections or medications.

The blood draw for bilirubin analysis should be performed the same day as the MAGIC assessment, except for the baseline measurement where sample collection is permitted within 48 hours before the randomization.

Both the Stage of each target organ and the overall grade will be documented in the eCRF to track disease progression.

Severe abdominal pain, ileus, and/or grossly bloody stool should be documented when present because Stage 4 lower GI-aGVHD is staged based upon the presence of these symptoms and is independent of volume of diarrhea. Streaks of blood in the stool due to hemorrhoids or anal fissures, or transient hematochezia following endoscopic biopsies are not considered when making this determination. The definition of severe abdominal pain remains a judgment call on the part of the treating clinician, but it is suggest only considering pain attributed to GVHD that requires the initiation of high doses of narcotic pain medication or a significant increase in on-going narcotic use, and the abdominal pain significantly impacts a subject's performance status as determined by the treating clinician [Harris, 2016].

As part of the analysis, MAP will be calculated. The exact date of recurrence of aGVHD and organ staging will be recorded in the eCRF.

Survival and disease progression (graft failure, FFS, NRM, OS, malignancy relapse and progression, GVHD response, and relapse) as well as GVHD relapse will be monitored throughout the trial. Date(s) of discharge, any readmissions, and hospital stays will be recorded. Where the status of the subject is unknown, and the subject fails to attend for a visit, attempts will be made to determine survival status including contacting the

subject's treating physician and (where contact details are available, and permission has been obtained) next of kin.

5.6 Parenteral Support

The average daily volume and caloric content of parenteral support used in the previous 7 days will be recorded in the eCRF.

5.7 Gastrointestinal Regeneration and Barrier Function

Gastrointestinal regeneration and barrier function will be assessed through plasma citrulline levels, mucosal cell population regrowth from biopsies, serum albumin, and incidence of systemic infections of likely GI origin.

5.8 Safety Parameters

5.8.1 Colonoscopy, Computed Tomography Colonography, or Magnetic Resonance Enterography

A colonoscopy must be performed at the screening, 1-year, and 2-year visits after apraglutide treatment to detect colonic polyps (Table 1 and Table 2). In general, a colonoscopy is the gold standard for polyp detection. Therefore, this should be the endoscopic procedure of choice whenever possible. Subjects with a low platelet count may receive a platelet transfusion prior to this procedure.

The population in this trial includes subjects with lower GI-aGVHD. These subjects have highly inflamed, friable, ulcerated, and denuded GI mucosa, with large volumes, frequently bloody, osmotic, and bile acid diarrhea. This places GI-aGVHD subjects at a higher risk of bleeding or perforation during a colonoscopy [Schwartz, 2001; Lohsiriwat, 2010]. Therefore, in this trial, the Investigator can, based on his/her judgment, perform a CT colonography (where allowed) or MR enterography as an alternative method for polyp detection. **In Germany**, only colonoscopies or MR enterography (not CT colonographies) can be performed.

At screening, historical data may be used if obtained within 6 months prior to the screening visit. Any polyps identified in the screening assessment should be removed. Histopathology assessment of any removed polyps must be documented in the source documents and in the eCRF. **Subjects who do not want to have their polyps removed or whose histology reports show malignancy cannot be randomized and dosed with apraglutide.**

5.8.2 Physical Examination

A general physical examination will be completed at the times outlined in the Schedule of Assessments (Table 1 and Table 2). Additional physical examination should be performed at any time during treatment, if clinically indicated.

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

5.8.3 Body Weight and Height

Body height will be recorded at screening to the nearest 0.5 cm. Subjects should be measured without footwear.

Body weight will be measured at each visit using the standard scales at the site, using the same scale at each assessment for a subject as far as possible and recorded in

local standard metrics. During weighing, the subject should wear light clothing and no shoes.

5.8.4 Vital Signs

Vital signs including systolic and diastolic blood pressure, heart rate, and temperature will be measured before apraglutide administration at the times outlined in Table 1 and Table 2.

Vital signs will be measured in a sitting or supine position after 5 minutes rest and blood pressure will be recorded in millimeters of mercury (mmHg). Temperature will be recorded in either degrees centigrade or degrees Fahrenheit.

Measures will be collected in the source documents and eCRF.

5.8.5 Twelve-lead Electrocardiogram

A 12-lead ECG will be recorded at rest at the times outlined in Table 1 and Table 2 using the standard equipment and procedure at the site. Additional ECG monitoring should be performed at any time during the treatment, if clinically indicated. All ECG parameters, including heart rate, pulse rate, QRS, and QT will be recorded. The QTcF will be calculated in the eCRF.

If screening and randomization occur on the same day, historical ECG results obtained within 48 hours can be used.

If randomization occurs within 48 hours from screening, the ECG does not need to be repeated.

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 after signing of the ICF which meet the definition of an AE must be recorded in the eCRF (see Section 6.5).

5.8.6 Adverse Events

Adverse events will be recorded in the AE pages of the eCRF for all eligible subjects. During screening only SAEs or trial related AEs will be reported. Once eligibility is confirmed all AEs will be reported in the ECRF. Adverse events will be reported using a recognized medical term or diagnosis that accurately reflects the event. Adverse events will be assessed by the Investigator for severity, relationship to the investigational product or a protocol requirement, possible etiologies, and whether the event meets criteria as an SAE and therefore requires immediate notification of the Sponsor. See Section 6.2 for the definition of SAEs, and Section 6.2.6 and Section 6.2.3 for guidelines for assessment of severity and relationship, respectively. If the event has not recovered to baseline or Grade 1 at the end of the trial reporting period, it will be documented as ongoing for all events that are at least possibly related to apraglutide. If an AE evolves into a condition which becomes “serious” it will be reported as an SAE.

Any laboratory abnormality will be considered an AE if it is judged to be clinically significant by the Investigator.

Infections (local as well as systemic) and sepsis events will be captured in the eCRF together with the severity and any interventions taken; they will be coded according to the version of the Medical Dictionary for Regulatory Activities (MedDRA) in use in the trial at the time.

5.8.7 Adverse Events of Special Interest

The handling and further clarification of AESIs are described in detail in Section 6.2.2; special reporting requirements apply (refer to Section 6.3.4). When documenting an AE as per the section above, the Investigator will assess if the AE is an AESI.

In this trial, AESIs are:

- Injection site reactions
- Gastrointestinal obstructions
- Gallbladder, biliary and pancreatic disease
- Fluid overload
- Colorectal polyps
- New malignancies
- Systemic hypersensitivity

5.9 Laboratory Analysis

The total blood volume to be collected in this trial will not exceed 550 mL for each subject. For subjects with a positive historical serology result post-transplantation for cytomegalovirus, Epstein-Barr virus, SARS-COV-2 virus, HIV, hepatitis B virus, or hepatitis C virus an additional volume, up to a maximum of 4 mL will be drawn for viral load assessment.

Information of the clinical laboratories involved in the trial will be available in the Laboratory Manual and Site File.

5.9.1 Local Laboratory Parameters

Blood samples for clinical chemistry, including liver enzymes, hematology, and hemostasis, and urine samples for urinalysis will be collected at the times outlined in Table 1 and Table 2.

Blood and urine samples will be processed at the site in accordance with local procedures. Local laboratory ranges for each site must be provided to the Sponsor prior to site initiation for the trial.

All local 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 (see Section 6.5).

The following clinical chemistry parameters will be collected

- Total HCO_3^-(bicarbonate)
- Calcium
- Phosphate
- Sodium
- Chloride
- Ferritin
- Glucose
- Blood urea nitrogen and/or urea
- Creatinine and estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI formula

Note: The CKD-EPI formula employs serum creatinine measurements and a subject's age, sex, and race to estimate the glomerular filtration rate.

An online calculator is available at: [https://www.merckmanuals.com/medical-calculators/GFR_CKD_EPI.htm].

The formula is: eGFR = 141 x min (serum creatinine/κ, 1)$^{\alpha}$ x max (serum creatinine/κ, 1)$^{-1.209}$ x 0.993^{Age} x 1.018[if female] x 1.159[if black]

The eGFR is expressed as mL/min/1.73 m^2 of body surface area, L; κ = 0.7 (females) or 0.9 (males); α = -0.329 (females) or -0.411 (males); min = indicates the minimum of serum creatinine/κ or 1; max = indicates the maximum of serum creatinine/κ or 1; age is in years. This formula expects creatinine to be measured in mg/dL.

- Uric acid
- Bilirubin (total, conjugated [direct] and, if possible, unconjugated [indirect])
- Lactate dehydrogenase
- Alkaline phosphatase
- Alanine aminotransferase
- Aspartate aminotransferase
- Albumin
- C-reactive protein
- Gamma-glutamyl transferase
- Total amylase and, if possible, pancreas type [pancreatic amylase]
- Triacyl glycerol lipase (lipase)

The following hematology parameters will be collected:

- Erythrocytes
- Hematocrit
- Hemoglobin
- Leukocytes, including differential counts (% and/or absolute values. However, to confirm inclusion criterion 4 absolute neutrophil count is required)
- Thrombocytes (platelets)
- International normalized ratio
- Hemostasis
- Prothrombin time and, if possible, activated partial thromboplastin time

The following urinalysis parameters will be collected:

- pH
- Leukocytes or leukocyte esterase
- Nitrite
- Protein
- Glucose
- Ketones

- Hemoglobin (blood)
- Specific gravity

Urinalysis parameters will be measured via dipstick or site-specific procedures.

5.9.1.1 *Assessment of Viral Load*

For subjects with a positive historical serology result post-transplantation for Epstein-Barr virus, cytomegalovirus, SARS-COV-2 virus, HIV, hepatitis B virus, or hepatitis C virus, viral load will be assessed by polymerase chain reaction.

A sample must be collected within 7 days prior to randomization/Day 0.

5.9.1.2 *Pregnancy Test*

Pregnancy tests (serum at screening and baseline, urine test thereafter) will be performed for all female subjects of childbearing potential at the times outlined in Table 1 and Table 2. A positive urine pregnancy test should be confirmed using a serum pregnancy test prior to deciding on subject discontinuation of trial medication or participation.

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; this may be confirmed with a follicle-stimulating hormone test if there is doubt). Any pregnancies should be reported as described in Section 6.3.5.

5.9.2 Central Laboratory Parameters

Anti-Drug Antibodies

A blood sample for analysis of anti-apraglutide antibodies that may include neutralizing antibodies and cross-reactivity to endogenous GLP-2 (collectively ADA) will be collected prior to dosing, at the times outlined in Table 1 and Table 2.

At Weeks 21, 52, and 104, samples will not be collected if treatment was stopped before the previous sample collection date and the previous sample result was negative (if these results are available). In addition, unscheduled samples should be collected in the case of an AE judged relevant by the Investigator, such as hypersensitivity.

Subjects who discontinue the trial prematurely will be asked to have ADA samples drawn at 4 (±2) and 8 (±2) weeks after an early trial discontinuation visit. The Sponsor will inform the Principal Investigator of these results once available.

Stool Sample Collection

Stool sample collection can be done within 24 hours prior to the visit.

Subjects will be asked to bring a stool sample to each visit outlined in Table 1 and Table 2, and samples will be frozen prior to shipment to the central laboratory for analysis of calprotectin and microbiome. Collection pots and instructions will be provided to subjects by site staff. Sample processing, storage, and shipment details will be outlined in the Laboratory Manual.

Biomarkers

Blood samples for analysis of biomarkers (including but not limited to albumin, citrulline, REG3α, ST2, angiopoetin-1 and -2, sTM, and VEGF) will be collected at the times outlined in Table 1 and Table 2. Different biomarkers may be tested at different time points.

Details on blood volume, stool samples, handling, storage, and shipment of the samples to the central laboratory will be provided in a trial-specific Laboratory Manual.

Lower GI Biopsy

The screening and Day 56 lower GI biopsy samples are optional and should be obtained only if the subject is medically suitable for the procedure. Historical lower GI biopsies collected at clinical diagnosis of lower GI-aGVHD are allowed to confirm eligibility (if a biopsy is needed to rule out other reasons for diarrhea) and further trial analysis.

If lower GI biopsy was not obtained at screening, the Day 56 lower GI biopsy is not required.

A **less invasive sigmoidoscopy** may be used for taking lower GI biopsies instead of full colonoscopy.

The analysis for mucosal cell regrowth assessment will be done at a central laboratory. Further details on the processing of biopsy samples are described in the Laboratory Manual.

Pharmacokinetics

Pre-dose Pharmacokinetic Samples

Blood samples for analysis of the plasma concentration of apraglutide will be collected at the times outlined in Table 1 and Table 2. Pre-dose PK samples must always be drawn within 2 hours before administration of apraglutide on Weeks 1, 2, 3, 4, 6, and 8. The exact time of PK sampling and subsequent apraglutide administration must be captured.

If the dose is not given, e.g., for safety reasons, the sample should however be collected at any time of the visit, and **the actual time should be documented.**

In cases where the apraglutide was not administered during the previous week, e.g., for safety reasons, the pre-dose PK sample at the current visit can be skipped.

Post-dose Pharmacokinetic Samples

Post-dose PK samples should be collected at Week 0 and Week 4 (or if not possible at Week 4, then PK sampling should be performed at Week 5 or Week 6 or Week 7 or Week 8).

The following post-dose PK samples are required at:

- 6 hours
- 30 hours
- 72 hours
- 120 hours
- 168 hours (this sample is not to be collected if a pre-dose sample is collected for the subsequent IMP administration)

All post-dose PK samples must be collected within ±2 hours window, except for the 6-hour post-dose sample where only ±1 hour window is permitted.

It is recommended to administer the IMP in the morning to avoid performing PK sample collection during the night.

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 IMP administration and PK sampling.

6 SAFETY

All AEs regardless of relationship to IMP will be collected, fully investigated, and documented in the source documents from ICF signature until EOT visit. For screen failures, AEs reporting is described in Section 2.2.8. The region-specific emergency contact details for the Sponsor can be found in the Investigator Site File (ISF).

After the Week 104/EOT visit, only SAEs that are judged as related to apraglutide will be reported.

In case of early trial discontinuation, AEs should be collected until 4 weeks after the early trial discontinuation date.

Adverse events that occur prior to first dosing will be captured as pre-treatment AEs.

6.1 Safety Data Reporting

The Investigator must monitor the condition of the subject throughout the trial from the time of obtaining informed consent until the last protocol-specific procedure, whether it is the EOT visit, or during the safety follow-up period.

If an Investigator becomes aware of an SAE after the subject's last visit (this includes withdrawn subjects), and he/she assesses the SAE to have a reasonable possible causality to the IMP, the case will have to be reported to the Sponsor via the Contract Research Organization (CRO) using the contact information, regardless of the length of time that has elapsed since the end of the trial.

6.2 Definition and Assessment of (Serious) Adverse Events and Other Safety Related Events

An AE is any untoward medical occurrence in a subject or a clinical investigation subject administered a pharmaceutical product, and which does not necessarily have a causal relationship with the trial procedure. 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 related to the medicinal (investigational) product or not International Council on Harmonisation ICH E6(R2) [ICH E6 (R2), 2016].

The sources of AEs include:

- The subject's response to questions about his/her health (a standard non-leading question such as "How have you been feeling since your last visit?" is asked at each visit)
- Symptoms spontaneously reported by the subject
- Investigations and examinations with findings that are assessed by the Investigator to be clinically significant
- Other information relating to the subject's health becoming known to the Investigator

Medical conditions present prior to signing the ICF are defined as MH and are not to be considered AEs.

If an AE or MH event worsens in severity or frequency during the trial, then a new AE is to be registered with the date of the worsening as the start date.

For surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

An SAE is classified as any untoward medical occurrence that:

- 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 in a neonate/infant born to a mother exposed to IMP (also applies if father was exposed to IMP)
- Is a clinically significant event in the Investigator's judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above) [ICH E2A, 1995]

6.2.1 Events that Do Not Meet the Definition of a Serious Adverse Event

Elective hospitalizations to administer or to simplify trial treatment or trial procedures are not considered SAEs. However, all events leading to unplanned hospitalizations or unplanned prolongation of an elective hospitalization (for example, undesirable effects of any administered treatment) must be documented and reported as SAEs.

Events that do not meet the definition of an SAE:

1. Hospitalization or prolongation of hospitalization as part of a routine procedure
2. Hospitalization for a survey visit, annual physicals or social reasons
3. Hospitalization that does not include an overnight stay
4. Elective hospitalizations for pre-existing conditions documented in the MH that have not worsened

In case of doubt, an event should be reported within timelines.

6.2.2 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 is 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.

Once the clinical database containing all AEs is available, potential AESIs will be identified using the following algorithmic standardized MedDRA queries (including Standardized MedDRA Queries [SMQs]):

- Standardized MedDRA Queries—functional, inflammatory- and gallstone-related biliary disorders
- Standardized MedDRA Queries—GI perforation, ulceration, hemorrhage, or obstruction
- Standardized MedDRA Queries—malignancies

In the absence of related SMQs, custom queries will be set up from the Sponsor-defined list of Preferred Terms (PTs) as listed in the Statistical Analysis Plan (SAP). These standardized searches will occur prior to each iSRC meeting.

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

Injection Site Reactions

Subjects will be monitored for ISRs for at least 1 hour after each 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 should be reported as AEs.

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

- Pain
- Erythema
- Induration
- Pruritus
- Bruising

Severity and duration of these features of the ISR will be collected by the site by direct observation when IMP is administered on site.

Table 8: 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 activities of daily living
Grade 3	Severe pain; limiting self-care activities of daily living
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 activity of daily living, and consideration of treatment interruption
Erythema	
Grade 1	Mild, <2.5 cm
Grade 2	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

Gastrointestinal Obstruction

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

Gallbladder, Biliary, and Pancreatic Disease

These should be monitored by symptoms, liver enzymes (ALT, AST, and alkaline phosphatase), bilirubin, lipase, and amylase, and subjects should be treated according to the Investigator's judgement.

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.

Colorectal Polyps

Colonoscopies or colonographies (where allowed) or MR enterographies will be performed during the trial (Table 1 and Table 2). Any polyps found during colonoscopies must be removed, and the histology documented in the source documents and eCRF. **In Germany**, only a colonoscopy or MR enterography (not colonography) can be performed.

New Malignancies

For any subject with a malignancy identified during the trial, a thorough MH 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 IMP treatment will be discontinued, and the subject will be closely followed up by the Investigator (subjects must be treated and followed up as per local guidelines and practices for those malignancies).

Systemic Hypersensitivity

Hypersensitivity and anaphylaxis (narrow SMQs) will be collected as AESIs.

6.2.3 Assessment of Causality

Both the Investigator and Sponsor will assess the causality of the event in relation to the IMP, based on the criteria listed in the International Council on Harmonisation ICH E6(R2) guidelines [ICH E6 (R2), 2016].

Investigators must also systematically assess the causal relationship of AEs to IMP(s)/trial treatment (including any other non-IMPs, radiation therapy, etc.) using the following definitions. Decisive factors for the assessment of 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, MH, concomitant medication, course of the underlying disease, and trial procedures.

Table 9: 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	Y	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

Not related: Not reasonably related to the IMP. The AE 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. The AE could medically (pharmacologically/ clinically) be attributed to the IMP under trial in this clinical trial protocol. Table 9 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.

6.2.4 Unexpected Adverse Drug Reaction

An “unexpected” adverse drug reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator’s Brochure for drugs that are not yet approved and Product Information for approved drugs, respectively) [ICH E2A, 1995].

6.2.5 Suspected Unexpected Serious Adverse Reactions

The Sponsor will evaluate any SAE that has been reported regarding seriousness, causality, and expectedness. If the event is related to the IMP and is both serious and unexpected, it is classified as a suspected unexpected serious adverse reaction (SUSAR).

6.2.6 Assessment of Severity

The Investigator is required to grade the severity or toxicity of each AE.

Investigators will reference the National Cancer Institute (NCI)–CTCAE [NCI-CTCAE, 2017], version 5 (publication date: 27 November 2017), a descriptive terminology that can be used for AE reporting.

A general grading (severity/intensity; hereafter referred to as severity) scale is provided at the beginning of the above referenced document, and specific event grades are also provided.

If a particular AE's severity is not specifically graded by the guidance document, the Investigator is to use the general NCI-CTCAE definitions of Grade 1 through to Grade 5 following his or her best medical judgment [NCI-CTCAE, 2017].

The five general grades are:

- Grade 1 or Mild
- Grade 2 or Moderate
- Grade 3 or Severe
- Grade 4 or Life-threatening
- Grade 5 or Death

6.3 Reporting of Serious Adverse Events and Other Safety Related Events

6.3.1 Reporting of Adverse Events

All AEs must be reported to the CRO and/or Sponsor through the eCRF for all eligible subjects and within the data entry timeline defined for this trial and stipulated in the site agreement.

6.3.2 Reporting of Serious Adverse Events

This trial will comply with all applicable regulatory requirements and adhere to the full requirements of ICH Topic E2A (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting) [ICH E2A, 1995].

All SAEs must be reported on both the clinical and safety databases immediately and within a maximum of **24 hours** to the CRO and/or Sponsor of the trial through the eCRF.

All SAEs occurring after signing of informed consent up to EOT must be reported on AE pages in the eCRF or on an SAE form (if the eCRF is not available), regardless of the Investigator-attributed causal relationship with trial medication or trial-mandated procedures.

The CRO/Sponsor will re-evaluate the SAE and queries will be raised in the eCRF 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 for other SAEs must be entered into the eCRF as soon as possible, within 24 hours after it becomes available. In case eCRF access is not possible, the Investigator must contact the Clinical Research Associate (CRA) in charge of the site and/or the medical person allocated to the site (contact names are available in the ISF).

Serious AEs resulting in death will be reported to the Independent Ethics Committee (IEC) **within 7 days or sooner based on local regulations.**

The minimum data required for a report is:

- Subject identification (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

6.3.3 Reporting of Suspected, Unexpected, Serious Adverse Reactions

A SUSAR for an event that is life-threatening or fatal needs to be reported to the IEC/Institutional Review Board (IRB) (local event via local Investigator) and competent authorities (CAs) within 7 calendar days.

All other SUSARs need to be reported to the IEC/IRB (local event via local Investigator) and CAs within 15 calendar days.

As necessary, the Sponsor must inform the iSRC and all Investigators, IEC/IRBs, and CAs participating in the clinical trial of the occurrence of a SUSAR.

6.3.4 Reporting of Safety Signals

All suspected new risks and relevant new aspects of known adverse reactions that require safety-related measures, i.e., so-called safety signals, must be reported to the Sponsor within 24 hours. The Sponsor must report the safety signals within 7 days to the IEC/IRB (local event via local Investigator) and CAs.

The Sponsor must immediately inform all participating Investigators about all safety signals. The other IECs/IRBs and CAs involved in the trial will be informed about safety signals by the Sponsor as necessary.

6.3.5 Reporting and Handling of Pregnancies

Pregnant subjects must immediately be withdrawn from the clinical trial. Any pregnancy, in a female subject or female partner of a male subject, identified during the treatment phase of the trial and within 90 days after discontinuation of trial medication must be reported to the Sponsor **within 24 hours** (in the case of a female partner of a male subject this will be within 24 hours after obtaining written informed consent from the pregnant woman). 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 must be instructed to stop taking the IMP and immediately inform the Investigator if she becomes pregnant. 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 and the child should continue until 4 weeks after the outcome of the pregnancy is known.

6.3.6 Periodic Reporting of Safety

An annual safety report will be submitted **once a year** to the local IEC/IRB via the local Investigator and to CAs via the Sponsor or CRO.

6.4 Follow-up of Serious Adverse Events

Serious AEs should be followed until resolution or stabilization. Subjects with ongoing SAEs at trial termination (including safety visit) will be further followed up until recovery, return to baseline status, or stabilization. If after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the eCRF SAE form.

6.5 Abnormal Laboratory Findings and Other Abnormal Investigational Findings

Abnormal laboratory findings and other abnormal investigational findings (for example, on an ECG trace) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation, or are considered otherwise clinically significant by the Investigator. If a laboratory abnormality fulfills these criteria, the identified medical condition must be reported as the AE rather than the abnormal value itself.

Elevations (or new elevations from baseline) in transaminases or tests of liver function will be closely monitored. Laboratory tests (AST, ALT, alkaline phosphatase, gamma glutamyl transaminase, total bilirubin, viral serologies) will be used to distinguish other causes of liver injury from drug-induced liver injury. Subjects with persistent or new deterioration of hepatic function will be followed up.

6.6 Trial Specific Safety Considerations

6.6.1 Overdose

Details of overdose of apraglutide or any concomitant therapy must be recorded in the eCRF and on the dedicated paper form to be included into the Safety Database. Any overdose that is considered by the Investigator to be clinically significant must be reported as an SAE in the eCRF.

6.6.2 Considerations on Investigational Medicinal Product Dose Reduction and Temporary Discontinuation

If the Investigator reduces the dose of the IMP because of documented safety reasons, the safety reasons should be documented as an AE.

Reduction of IMP means reducing the dose by one step (see Table 5) or pausing in case of general tolerability issues. The IMP dose can only be reduced by one step during the trial.

For specific dose adjustments of apraglutide in cases of abnormal liver function tests, bilirubin, or other clinical chemical parameters, refer to Section 4.2. **The Investigator should attempt to return to the per protocol dose as soon as the medical condition of the subject allows and a new weekly schedule of IMP administration may be established.** If the subject does not continue to tolerate the per protocol dose the Investigator may choose to resume IMP administration at the weekly lower dose.

Changes and discontinuations must be reported to the CRA in charge of the site and discussed with the Medical Monitor. If the subject's medical condition allows, this discussion may occur before any dose change/discontinuation but must occur after if not possible (e.g., an AE necessitating an immediate stop to dosing with apraglutide). The final decision to reduce or discontinue IMP rests with the Investigator.

Dose changes and the rationale will be documented in the medical records, and in the eCRF. Missed doses (scheduled doses that have not been taken) will not be made up for, i.e., the treatment period will not be prolonged due to temporary discontinuation. Missed doses should be reported as a protocol deviation.

6.6.3 Trial Holding/Stopping Criteria

The trial will be placed on hold (i.e. screening or randomization of new subjects and treatment of existing subjects should be interrupted) if, during the first 182 days after the start of apraglutide treatment, a subject experiences:

- Emergence of a new GI or hepatobiliary cancer

or

- Undergo major surgical intestinal resection due to a proliferative intestinal phenomenon

Subsequently, an ad-hoc iSRC will review the data and make a recommendation to either continue or stop the trial.

The following toxicity events have been identified for monitoring:

- Apraglutide-related (definitely, probably, possibly) deaths
- Grades 3–4 apraglutide-related (definitely, probably, possibly) organ toxicities
- Any apraglutide or/and RUX administration stop or hold due to elevation of ALT/AST and/or bilirubin as defined in Section 4.2.1.2 and Section 4.2.2.1 (confirmed Hy's law cases)

These events will be monitored in accordance with the Toxicity Guidance Rule outlined in Table 10. If the Guidance Rule is met, this will trigger an iSRC review to re-evaluate the benefit risk of apraglutide and thus make recommendations on the further conduct of the trial as necessary and appropriate. During a triggered iSRC review, apraglutide dosing to existing subjects, and the screening/randomization of new subjects into the trial, will be suspended until the iSRC has concluded their review.

Table 10: Toxicity Guidance Rule

Number of subjects randomized	Number of observed toxicity events triggering iSRC meeting
1–6	≥1
7–12	≥2
13–15	≥3
16–21	≥4
22–24	≥5
25–27	≥6
28–30	≥7

iSRC=independent safety review committee

7 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

7.1 General Considerations

All continuous efficacy data will be summarized descriptively by dose group and time point including changes from baseline (n, arithmetic mean, standard deviation, minimum, lower quartile [Q25], median, upper quartile [Q75], and maximum). Categorical variables will be summarized with frequencies and percentages by treatment group and time point.

From an efficacy perspective, the overall statistical aim of the trial is geared toward estimation. Thus, efficacy data collected on both the low and high doses will be compared to external data on BAT where possible using Bayesian methods to provide insight into the likelihood of efficacy vis-à-vis BAT. In this sense, statistical analysis will not focus upon p-value generation but rather estimation and quantification of uncertainty.

A SAP will be prepared and finalized prior to the final analysis. The SAP will detail the implementation of the planned statistical analyses in accordance with the principal features stated in the protocol, and will provide full details of the analyses, data displays, and algorithms to be used for data derivation.

The final analysis will be done in two steps. The primary analysis of safety and efficacy will be performed once all subjects have either completed Day 91 or withdrawn from the trial. Additional analyses will be done once all the subjects have either completed the EOT visit or withdrawn from the trial. A CTR will be issued after the primary analysis. An addendum to the CTR will cover the follow-up period until 2 years after the first apraglutide dose.

7.2 Interim analysis

An interim analysis will be performed when 17 subjects have either reached Day 56 or withdrawn from the trial. All available data points by the data cut-off date will be included in the analysis. This interim analysis does not represent a typical interim analysis with testing of hypotheses and alpha spend.

7.3 Missing, Unused, and Spurious Data

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

7.4 Determination of Sample Size

The principal statistical goal of this trial from an efficacy perspective is to estimate the likelihood of efficacy of apraglutide therapy in the treatment of subjects with SR lower GI-aGVHD after alloSCT. Published, randomized control trial data on BAT in the treatment of such subjects [Zeiser, 2020a] provides estimates of overall response rate at Day 28 of 62% (96/154) and durable overall response at Day 56 of 40% (61/154). Further, the GI-aGVHD response rate at Day 28 of 57% (55/96) is also published Zeiser, 2020b]. At the time of trial initiation, this was considered the best matched information available for a reasonable sample size determination.

Assuming same degree of decline of GI response from Day 28 to Day 56 as observed for an overall response from Day 28 to Day 56, the GI response at Day 56 can be estimated as 36% (r=35 responses in n=96 subjects). These data provide a reliable prior for the true response rate on BAT, π_r, such that $\pi_r \sim Beta(r+1, n-r+1)=Beta(36,62)$."

In the current trial, it is planned to randomize 30 subjects on a 1:1 basis to high or low dose apraglutide. Assuming a non-informative prior for the Day 56 GI response rate with apraglutide, 15 subjects per dose will provide a posterior distribution for the true response rate with apraglutide π_a, such that π_a~Beta(s+1,15-s+1) where s is the observed number of successes on any given dose. Using the prior for BAT response, π_r, and the posterior for the apraglutide response, π_a, it is possible to construct the posterior distribution for the true difference in response rates, π_a-π_r.

Considering a penalty on the original assumptions that are based on Day 56 durable overall response instead of Day 56 response, one can still detect an effect of apraglutide over BAT.

At least five out of seven responders at the interim analysis will be able to detect a signal of apraglutide at least 10% greater than the Day 56 durable overall response with a probability of 88.2%.

In case of a sufficiently large response rate in a single treatment arm, the planned sample size will allow for reliable conclusions regarding the effectiveness of apraglutide relative to BAT for Day 56 response rate in the treatment of subjects with SR lower GI-aGVHD after alloSCT. Considering a penalty on the original assumptions, we can still detect an effect of apraglutide over BAT.

In addition to the N=30 subjects randomized to receive low or high doses of apraglutide once weekly for 26 weeks, a separate, non-randomized cohort of up to four subjects with body weights ranging from 40.0 to <50.0 kg will be assigned to receive 2.5 mg apraglutide (LBW dose).

7.5 Definition of Analysis Sets

7.5.1 Full Analysis Set

The Full Analysis Set (FAS) comprises all *enrolled subjects (*randomized *and non-randomized LBWs)*. All efficacy evaluations will be conducted using the FAS according to the planned dose (high dose, low dose, LBW dose) and in total. All subjects will be analyzed according to the apraglutide dose assigned by randomization.

7.5.2 Low Body Weight Analysis Set

The LBW Analysis Set comprises a separate, non-randomized cohort of up to four subjects with body weights ranging from 40.0 to <50.0 kg assigned to receive 2.5 mg apraglutide (LBW dose) who receive at least one dose of therapy and who provide at least one post-baseline assessment for any secondary endpoint.

7.5.3 Safety Analysis Set 1

The Safety Analysis Set (SAS) 1 (SAS1) comprises all randomized subjects exposed to both apraglutide dose levels. All principal safety analyses of subjects randomized low or high doses of apraglutide will be carried out on the SAS1. Subjects are analyzed according to the dose of apraglutide received.

7.5.4 Safety Analysis Set 2

The SAS2 comprises all randomized and non-randomized subjects exposed to both apraglutide dose levels. Supportive safety analyses will be carried out on the SAS2. Subjects are analyzed according to the dose of apraglutide received.

7.6 Statistical Evaluation of Safety

Safety data will be summarized by randomized dose separately and combined for both SAS1 and SAS2. Safety data on apraglutide 2.5 mg in the non-randomized LBW Analysis Set will be listed and presented separately. Safety assessments include AE reporting, vital signs, 12-lead ECG, clinical laboratory evaluations, and physical examinations.

7.6.1 Adverse Events

All AESIs, treatment-emergent AEs (TEAEs), SAEs, AEs leading to discontinuation, AEs leading to death and laboratory abnormalities will be summarized by apraglutide dose received. Adverse event data will be coded using the latest version of MedDRA. A definition of TEAEs will be presented in the SAP. Treatment-emergent AEs will be summarized descriptively by apraglutide dose received, and by the frequency of subjects experiencing events corresponding to body systems and MedDRA PT.

Subjects with multiple occurrences of events will only be counted once at the maximum severity/grade to trial therapy for each PT, SOC, and overall. Should the majority of subjects experience multiple occurrences of the same event type, individual event rates of these events will be evaluated.

A summary table will be presented for each of the following AE categorizations in terms of the number and percentage of subjects experiencing AEs by SOC and PT:

- All TEAEs
- Serious TEAEs
- Treatment-emergent AEs by severity (mild/moderate/severe)
- Treatment-emergent AEs leading to withdrawal from the trial
- Treatment-emergent AEs leading to discontinuation of randomized treatment
- Treatment-emergent AEs leading to death

Furthermore, AEs will be summarized by causality and the maximum intensity. Any SAEs and/or AEs that led to withdrawal will be listed. All AEs will also be summarized by relatedness to apraglutide therapy. Adverse events that are reported as possibly, probably, or definitely related to apraglutide therapy will be counted as related to trial therapy. Adverse events with a missing relationship will be considered as “related” for this summary.

The SAP will provide detailed descriptions for the analysis of other AE information, such as duration, action taken, and clinical outcome.

7.6.2 Adverse Events of Special Interest

The individual and cumulative incidence of the following groupings AESIs will be summarized by dose:

- Injection site reactions
- Gastrointestinal obstructions
- Gallbladder, biliary, and pancreatic disease
- Fluid overload
- Colorectal polyps
- New malignancy
- Systemic hypersensitivity

The specific AE PTs falling into each of these groupings will be determined prior to randomization of the first subject and will be captured in the SAP.

7.6.3 Laboratory Results

Hematology and chemistry laboratory data will be summarized by apraglutide dose received. Clinical Laboratory evaluation results will be listed and compared to laboratory reference ranges, with those values outside of the applicable range flagged as high or low. Shift tables will be provided to examine the distribution of changed pre/post receipt of apraglutide therapy. Quantitative laboratory data will be summarized using descriptive statistics (n, arithmetic mean, median, minimum, and maximum) for each parameter by dose and time.

7.6.4 Vital Signs

Each vital sign and respective change from baseline will be summarized over time by apraglutide dose received. Subjects with clinically significant abnormalities in vital signs as compared to baseline will be listed.

7.6.5 Physical Examination

A summary table for the shift from pre-/post-trial therapy received in physical examination will be summarized over time by apraglutide dose.

7.6.6 Twelve-Lead Electrocardiogram

The ECG data will be summarized by apraglutide dose received over time using descriptive statistics for change-from-baseline values and categorical outliers. Any clinically significant changes will be recorded as AEs.

7.6.7 Anti-Drug Antibodies

All immunogenicity analyses will be descriptive and carried out with a special focus on neutralizing antibodies, and treatment-induced or treatment-boosted ADA. Impact on PK, PD, and safety will be described.

7.7 Pharmacokinetic Statistical Analysis

Apraglutide individual subject concentrations will be summarized in the FAS and the LBW Analysis Set. The PK parameters will be obtained as post-hoc estimates based on the population PK/PD analysis developed by Lyo-X. They will be reported in a dedicated separate report.

7.7.1 Population Pharmacokinetic Analysis

Apraglutide PK parameters: absorption rate constant (ka), apparent clearance (CL/F), and apparent volume of distribution (Vz/F) of apraglutide, with their intra- and inter-individual variability in the subject population, will be derived by population PK data analysis using a nonlinear mixed effects modeling approach, based on sparse sampling. These parameters will be reported in a dedicated separate report.

Trough plasma concentrations (C_{trough}) will be collected before dosing at Weeks 1, 2, 3, 4, 6, and 8. At Week 0 and Week 4 (or if not possible at Week 4, then samples should be collected at Week 5 or Week 6 or Week 7 or Week 8), samples will be collected at 6, 30, 72, 120, and 168 hours post-dose. Samples will have to be taken within ±2 hours window, except for the 6-hour post-dose sample where samples will have to be taken within ±1 hour window.

For every subject, visual inspection of the actual concentrations of apraglutide will be compared with a simulated PK profile based on a PK model developed on healthy volunteers and SBS patients' data from Phase I and II clinical studies. The simulated PK profile will be constructed based on the dose received and the subject's body weight. This will be performed online for every subject, and this will be used to check if the subject is correctly exposed to apraglutide.

7.8 Statistical Evaluation of Efficacy

In general, efficacy data will be summarized by randomized dose separately and combined in the FAS. Efficacy data on apraglutide 2.5 mg in the non-randomized LBW Analysis Set will be listed and presented separately.

7.8.1 Lower GI-aGVHD Response at Day 56

Overall response rate (PR and CR) at Day 56 on the lower GI tract MAGIC score was used for sample size estimation. It will be summarized descriptively and analyzed formally using Bayesian methods.

The prior for the true Day 56 lower GI response rate on BAT, π_r, and i will be based on the best matched historical control available at the time of analysis. The true response rate on BAT will be given, such that π_r~Beta(r+1,n-r+1), where n is the number of subjects treated with BAT and r is the number of successes observed in the historical control. Assuming a non-informative prior for the Day 56 lower GI response rate with apraglutide, the posterior distribution for true response rate with apraglutide 5 mg, π_a, will be given as π_a~Beta(s+1,m-s+1) where m is the number of subjects randomized to the 5 mg dose and s is the number of successes observed on this dose. A similar formulation applies to the 10 mg dose and for the two doses combined.

For each dose, and for doses combined, the response rate for BAT (π_r) and apraglutide (π_a) will be taken as the mean of the respective Beta distributions and the 5^{th} and 95^{th} percentiles will provide the associated 90% credible intervals. The difference in response rates, π_a-π_r, for each dose, and for the doses combined, will be taken as the difference in mean response rates. The associated 90% credible interval will be computed by sampling 100,000 pairs of random deviates from the respective Beta distributions, taking the difference of each pair (apraglutide – BAT) and extracting the 5^{th} and 95^{th} centiles of the resulting empirical distribution of the differences. Similarly, the probability that the true response rate is higher with apraglutide than with BAT will be taken as the fraction of differences greater than zero.

Day 56 lower GI response will also be summarized descriptively in the LBW Analysis Set using the Beta distribution. The response rate and associated 95% credible interval will be estimated and presented.

Subjects with missing data such that the Day 56 lower GI response cannot be determined will be included in the preceding analysis as non-responders consistent with intent to treat philosophy. A sensitivity analysis will also be performed based only on those subjects with non-missing data being consistent with a missing at random assumption.

To evaluate the robustness of the analysis of the Day 56 secondary endpoint, sensitivity analyses evaluating alternative response definitions taking account for the potential influence of intercurrent events like increase in steroids, development of infectious colitis, relapse, and the use of budesonide or other non-absorbable steroids will be made. Details of these analyses will be outlined in the SAP.

7.8.2 Other Secondary Endpoints

Secondary endpoints will be summarized descriptively in the FAS. These endpoints are listed in Section 1.5.2.2.

Secondary endpoints assessed as binary responses will be summarized and analyzed in the same fashion as outlined above; if prior data for BAT are not available for a given time point, then only the apraglutide data will be presented.

Subjects with missing data such that response cannot be determined will be included in the analyses as non-responders. Again, supportive analyses of the secondary endpoints will be performed based only on those subjects with non-missing secondary endpoint data consistent with a missing at random assumption.

Secondary endpoints assessed as time-to-event endpoints will be summarized using Kaplan-Meier analysis. The median time duration of response will be presented where possible, along with the range and the associated 90% confidence interval. For comparison to BAT where prior data exist on duration of response, the distribution duration of response will be modelled via the inverse gamma distribution and thus compared on a Bayesian basis.

Where there are competing risks, time to event data will also be summarized using cumulative incidence. For endpoints like graft failure and NRM, subjects who die prior to reaching either endpoint will be right censored at the time of death. For comparison to BAT where prior data exist, for example for FFS, the distribution of mean time to event will be modelled via the inverse gamma distribution and thus compared on a Bayesian basis. Similarly, the distribution of event rates will be modelled via a gamma distribution and compared on a Bayesian basis.

Further details of these analyses will be outlined in the SAP.

7.8.3 Exploratory Endpoints

Exploratory endpoints will be summarized descriptively in the FAS; full details will be provided in the SAP. These endpoints are outlined in Section 1.5.2.3.

7.9 Reporting Deviations from the Statistical Plan

Any deviations from the original statistical plan will be reported in the CTR with a rationale for the deviation.

8 INDEPENDENT SAFETY REVIEW COMMITTEE

Intensive safety monitoring will occur during the dosing of the first six subjects who weigh ≥50.0 kg. Following the randomization of the first subject, an iSRC will convene approximately every 4 weeks to review all available data and assess safety and tolerability. The iSRC may receive periodic updates between these planned meetings for continued monitoring and assessment if more frequent monitoring by the iSRC is required. These reviews will continue until the sixth subject has completed Day 56, at which time the iSRC will evaluate if 24 additional subjects, who weigh ≥50.0 kg, will be randomized to the two treatment arms. Randomization of these additional subjects can occur while iSRC monitoring is ongoing. Safety data from subjects <50.0 kg will also be evaluated by the iSRC as they are enrolled and being treated.

Assuming no issues are identified in the iSRC reviews of the initial six subjects, subsequent iSRC reviews are anticipated to occur when approximately 14, 22, and 30 subjects have completed Day 56 or withdrawn from the trial, whichever comes first. Details on the composition, conduct, tasks, and responsibilities of the iSRC will be described in a separate iSRC charter.

9 TRIAL CONDUCT

Written approval will be obtained from the IEC/IRB/CA concerning the conduct of the trial by the Sponsor before the trial commences. The clinical trial can only begin once approval from all required authorities has been received.

Steps to ensure the accuracy and reliability of data include the selection of qualified Investigators and appropriate trial sites, review, and training of protocol procedures with the Investigator and associated personnel before the trial, periodic monitoring visits, and thorough data management review.

9.1 Ethical Conduct of the Trial

This clinical trial will be conducted in compliance with this protocol and with the following:

- The ethical principles stated in the locally valid version of the Declaration of Helsinki
- All applicable laws and regulations of the country where the trial is conducted
- Applicable Good Clinical Practice (GCP) Guidelines (April 1996 ICH Guidance for Industry E6 GCP (including archiving of essential trial documents) and the Integrated Addendum to ICH E6(R2)
- The US FDA guidelines along with applicable laws and regulations including the FDA GCP Code of Federal Regulations (CFR) Title 21 [21 CFR]
- Clinical trial regulatory framework in the EU
- Any update to these regulations which occur during the conduct of the trial

9.2 Sponsor and Investigator Responsibilities

This trial will be conducted under a Clinical Trial Agreement between VectivBio ("Sponsor") and the institution(s) representing the investigational trial site(s) ("Authority"). Financial support to the investigational site(s) will be detailed in the Clinical Trial Agreement. The Clinical Trial Agreement must be signed before the commencement of the trial and will clearly delineate the responsibilities and obligations of Investigator and VectivBio ("Sponsor") and will form the contractual basis under which the clinical trial will be conducted.

The Sponsor is obligated to conduct the trial in accordance with strict ethical principles (Section 9.1). The Sponsor reserves the right to withdraw a subject from the trial (Section 2.2.6), to terminate participation of a trial site at any time, and/or to discontinue the trial early (Section 2.3).

The Sponsor agrees to provide the Investigator with sufficient material and support to permit the Investigator to conduct the trial according to the trial protocol.

By signing the Investigator Signature Page (found at the start of this protocol), the Investigator indicates that he or she has read the protocol carefully, fully understands the requirements and agrees to conduct the trial in accordance with the procedures and requirements described in this protocol.

The Investigator also agrees to conduct this trial in accordance with the laws, regulations, and guidelines outlined in Section 9.1. 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.

9.2.1 Ethical Considerations

Before trial initiation, this protocol, the proposed subject information and ICF, as well as other documents required by current regulations will be submitted to an IEC/IRB responsible for providing approval for the site.

A signed and dated statement that this protocol and the ICF have been approved by the IEC/IRB must be filed in the ISF and the Trial Master File. In accordance with GCP and applicable regulatory requirements, the trial will not start at a site before receival of the respective IEC/IRB approval that must be signed and dated.

Investigators are responsible for promptly informing the IEC/IRB and the authorities of all protocol amendments, serious adverse reactions, and SUSARs 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/IRB. In the case that a vulnerable subject enters the trial, the Investigator is encouraged to consult their local IEC/IRB for guidance.

The protocol may not be modified without written approval from the Sponsor. Protocol modifications or changes may not be initiated without prior written IEC/IRB/CA 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/IRB and written verification that the modification was submitted should 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 e.g., 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 IMP used in the trial. Documentation of IEC/IRB approval must be sent to the Sponsor immediately upon receipt.

The constitution of the IEC/IRB must meet the requirements of the participating countries and ICH E6(R2). A list of the IEC/IRB members with names and qualifications plus a statement that it is organized according to ICH-GCP (R2) and the applicable laws must be provided to the Investigator and to the CRO for filing and archiving as per applicable local law.

9.2.2 Investigator Delegation Responsibilities

While delegation of certain aspects of the trial 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 and/or 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 should 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 and IEC/IRB/CA. The Investigators are responsible for keeping an up-to-date ISF, which includes all required and relevant trial documents.

9.2.3 Competent Authorities and Regulatory Considerations

The clinical trial protocol and related documents will need CA approval or notification, as required by local regulations and requirements prior to the initiation of the trial, i.e., screening or enrollment/randomization of a subject. No changes will be made to the protocol or related documents without prior approval by the CA, except for non-substantial amendments which may be implemented with notification only. Annual reporting will be done, and early or planned trial end as well as the CTR will be submitted to the CA as required within the appropriate timeframe (e.g., early termination of the trial within 15 days and CTR within 1 year after last subject's last visit, respectively).

9.2.4 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 CA/IECs/IRBs. Investigators are responsible for providing information on financial interests during the trial and for 1 year after completion of the trial or for a longer period of time if required by local legislation.

9.2.5 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 their 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 (this will be in the form of a paper ICF or an electronic consent form) describing the trial and providing sufficient information for 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 process with a subject, using the current approved consent form, must be obtained before any trial specific procedures are conducted on the subject.

The written informed consent and assent documents should be prepared in the language(s) of the potential subject population, based on an English version provided by the Sponsor and should be easy to understand. Before a minor subject's participation in the trial, the Investigator is responsible for obtaining written informed consent from the parent or legal guardian and, in cases where institutional guidelines and the subject's age dictate, informed assent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the trial and before any protocol specific screening procedures or any trial drugs are administered. Sufficient time must be given to consider whether to participate in the trial. The acquisition of informed consent/assent and the parent/legal guardian's/subject's agreement or refusal should be documented in the subject's medical records, and the

informed consent/assent form(s) should be signed and personally dated by the parent/legal guardian/subject and by the trial person who conducted the informed consent/assent discussion. In general, permission should be obtained from both parents before a minor is enrolled. Permission must be obtained from both parents, unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child. The original signed informed consent/assent form(s) should be retained in the ISF and in any other locations required by institutional policy, and a copy of the signed consent/assent form(s) should be provided to the parent or guardian.

The subject should read and consider the statement before signing and dating the ICF that meets the requirements of 21 CFR Part 50 [21 CFR], local regulations, ICH guidelines, requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA), where applicable, and the IEC/IRB or trial site requirements, and should be given a copy of the signed document. The consent form must also be signed and dated by the Investigator (or their 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 IEC/IRB approved amendment to the ICF.

Subjects who are re-screened will undergo the consenting processes again and sign a new ICF prior to the re-screening procedures beginning.

9.2.6 Subject Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this trial must, where applicable, comply with HIPAA. A US-based site that is not a Covered Entity as defined by HIPAA must provide documentation of this fact to the CRO/the Sponsor.

VectivBio, as Data Controller, ensures that all processing activities involving personal data performed in the scope of this trial are compliant with, but not limited to, the requirements set by EU General Data Protection Regulation (GDPR 679/2016), its subsequent amendments, and any additional national laws on Data Protection, recommendations, and guidelines as applicable.

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 sites, Investigators, their staff, the Sponsor(s) and its designees involved in the trial. 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 will review their medical records and data collected during the trial. These records and data may, in addition, be reviewed by others including the following: monitors and 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 IEC(s)/IRB(s) 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.

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 archived by the Sponsor or its designee for a minimal period of 25 years.

Even though subjects are assigned a unique identifying number; age, sex, and birth year may also 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 age and birth year—will be recorded. Subject to adequate safeguards, 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, but not be limited to, to support regulatory submissions, to conduct new data analyses to publish or present the trial results, to answer questions asked by regulatory or health authorities, or for activities that are otherwise connected to the trial.

9.3 Trial Documents

All documentation and materials provided by Sponsor for this trial are to be retained in a secure location and treated as confidential material.

9.4 Early Termination of the Trial

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

Ethical concerns

- Insufficient subject recruitment
- When the safety of the subjects is at risk (refer to both Individual subject stopping rules and trial stopping criteria are described in Section 4.2.1.2 and Section 6.6.3, respectively)
- Alterations in accepted clinical practice that make the continuation of a clinical trial unwise
- Early evidence of benefit or harm of the experimental intervention

At the time of early termination of the trial, all subjects (regardless of their response to apraglutide) will receive GVHD treatment according to the established local standard of care.

9.5 Trial Site Closure

At the end of the trial, all trial sites will be closed. Sponsor may terminate participation of a trial site at any time. Examples of conditions that may require early 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/randomization
- Discontinuation of the trial, as decided by the Sponsor
- Institution and/or Investigator breaches the Clinical Trial Agreement, or the agreement is terminated
- Trial is not initiated or suspended at the institution for any reason

9.6 Protocol Amendments

The protocol may not be modified without written approval from the Sponsor. Protocol modifications or changes may not be initiated without prior written IEC/IRB/CA approval except when necessary to eliminate immediate hazards to subjects or when the changes involve only logistical or administrative aspects of the trial. Such modifications will be submitted to the IECs/IRBs/CA and written verification that the modification was submitted should be obtained.

If an amendment to this protocol is required, it will be classified into one of the following categories:

- Substantial Amendments are those considered “substantial” to the conduct of the clinical trial and are likely to have a significant impact on, e.g., 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 IMP used in the trial
- Non-substantial Amendments are amendments that are not considered to meet the definition of substantial

Investigators are responsible for promptly informing the IECs/IRBs of any amendments (substantial only in the EU) to the protocol.

Documentation of IEC/IRB/CA approval must be sent to the Sponsor immediately upon receipt.

All instances where the requirements of the trial protocol were not complied with will be tracked. Corresponding subjects may be withdrawn from the trial at the discretion of the Investigator and/or Sponsor. Trial protocol deviations arise when subjects who have been entered in the trial deviate from the IEC/IRB-approved trial protocol.

If a major protocol deviation (i.e., a deviation that could have a significant effect on the subject’s safety, rights, or welfare and/or on the integrity of the trial data) occurs, the Investigator must notify the Sponsor and the appropriate IRB/IEC as soon as possible or as per local requirements.

9.7 Contingency Measures

9.7.1 Clinical and Ancillary Supply Provisioning

Subjects will have drug administered exclusively during trial visits (details are described in the pharmacy manual).

10 DATA HANDLING AND RECORD KEEPING/ARCHIVING

10.1 Confidentiality and Access to Source Data

All documentation and materials provided by Sponsor to the investigational site for this trial are to be retained in an ISF, which should 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.

10.2 Record Keeping/Archiving

Each clinical site will retain in a secured location, trial records, and documents pertaining to the conduct of this trial and the distribution of 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 should be provided to VectivBio prior to transferring any records to another party or moving them to another location.

10.2.1 Storage of Biological Material and Related Health Data

Pharmacokinetics, ADA, neutralizing antibodies, and citrulline samples will be kept for a maximum of 15 years after the completion of the bioanalytical report to use later for refinement and optimization of the bioanalytical methods or for additional research involving apraglutide, GLP-1/2 biology and gastroenterology to make informed decisions as applicable to optimize product use and further development. Future analyses will not include any genetic testing but may include additional evaluation of biomarkers related to the trial population or antibody responses induced by apraglutide.

Tissue slides and blocks will be kept for a maximum of 3 months after completing the bioanalytical report. The tissue blocks will be either destroyed or returned to the sites. No future analysis is planned.

10.3 Case Report Forms

All subject data relating to the trial will be recorded using an eCRF (conforming to 21 CFR Part 11 requirements [21 CFR]) unless transmitted to the Sponsor or designee electronically (e.g., laboratory data).

The subjects screened into the trial will be identified in a clinical trial database by a subject number. The Investigator or delegate will enter subject data from the source documents into the eCRF.

Access to the eCRF is restricted to staff participating in the trial and the extent of access will depend on the subjects' user role in the trial. Following training, the trial staff will be

given access to the eCRF and be able to enter data. The eCRF is designed to capture all required information in compliance with applicable ICH-GCP standards.

The clinical database will be designed based on the final protocol, system configuration, and consistency check specifications.

A complete electronic audit trail will be maintained.

Medidata Rave will be used as an eCRF.

All eCRF data is to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

All sections of the eCRF are to be electronically approved by the Investigator or designee (i.e., to confirm the accuracy of the data recorded) using an electronic signature [21 CFR] after the data has been entered and all queries have been resolved, signifying that the data entered in the eCRF is complete and accurate. Any subsequent changes to any eCRF page require a new approval signature.

Data Collection

The Investigator or delegate is responsible for ensuring the accuracy, completeness, and timeliness of the data reported. A trial monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Data entry, corrections, and addressing of discrepancies will be made by qualified site personnel.

Subject screening and randomization data will be completed for all subjects (i.e., eligible and non-eligible) through the IRT system and eCRF.

For each subject enrolled/randomized, regardless of trial medication initiation, an eCRF must be completed and signed by the Investigator/delegate. This also applies to those subjects who fail to complete the trial. If a subject withdraws from the trial, the reason must be reported on the eCRF.

Clinical Data Management

Data are to be entered in the eCRF database in a timely manner of the subject's visit in accordance with the trial specific data management plan and according to the agreement between the Sponsor/CRO and the clinical trial site. Quality Control and data validation procedures are applied to ensure the validity and accuracy of the clinical data.

The Investigators will have access to the site eCRF data until the database is locked. Thereafter, they will have read-only access until sites receive the subjects' CRFs in PDF format via electronic means.

The eCRF must be kept current to reflect subject status at any time point during the trial.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. While entering the data, the Investigator or delegate will be instantly alerted to data queries by validated programmed checks. Additional data review will be performed by the Sponsor, by the CRA, or other CRO personnel on an ongoing basis to look for unexpected patterns in data and for trial monitoring. If discrepant data are detected, a query specifying the problem and requesting clarification will be issued and visible to the Investigator or delegate via the eCRF. All electronic queries visible in the system either require a data correction (when applicable) and a response from the Investigator or delegate to clarify the queried data directly in the eCRF, or simply a data correction in the eCRF.

This process will continue until database lock.

The Investigator or 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 CA queries, it is also necessary to have access to the complete trial records, provided that subject confidentiality is protected.

All AEs will be coded according to the latest version of MedDRA used by the Sponsor.

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 CRO Quality System documents. After database lock, the Investigator will receive the eCRFs of the subjects of his/her site (including the audit trail) on electronic media.

10.4 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 the 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 [FDA, 2013].

All entries into the eCRF performed by site personnel must be verifiable by source documents. Additionally, the following data entered in the eCRF should 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 system should be supported by corresponding source documentation, which is attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry which must remain readable, and should be explained if necessary (e.g., via an audit trail). Examples of acceptable source documentation include, but are not limited to, hospital records, paper diary, 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. 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 for which a risk-based approach will be taken. 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.

11 QUALITY ASSURANCE AND QUALITY CONTROL

This trial will be performed in compliance with the Clinical Trial Protocol, the Declaration of Helsinki, ICH E6(R2), and applicable regulatory requirements.

The accuracy, consistency, completeness, and reliability of the trial data produced under this protocol will be assured through quality assurance and quality control activities performed in accordance with the SOPs of the Sponsor or of the Sponsor representative (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.

11.1 Audits and Inspections

The trial may be subject to audit by the Sponsor or its designee, IEC/IRB, and/or CA inspections. Audits may be performed to check compliance with ICH E6(R2) guidelines and other applicable regulations, and may include:

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

The Sponsor or its designee may conduct additional audits on a selection of trial sites, which may require 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 IEC/IRB/CA inspections according to ICH E6 (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 CA informs the Investigator that it intends to conduct an inspection, the Investigator shall notify the Sponsor immediately.

11.2 Monitoring

The Sponsor and/or its representatives will conduct on-site monitoring visits to assess site qualifications and its appropriateness for inclusion in the trial, at the site initiation visit (SIV), and to monitor the trial to ensure compliance with the protocol, GCP, and applicable regulations and guidelines. Remote monitoring may be done, if needed. The frequency of the monitoring visits will be based on subject recruitment rate, data quality performance of site, and critical data-collection times. 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.

Before trial start, a SIV will be performed after the site is approved for inclusion in the trial by the Sponsor and essential approvals and documents are in place. The Principal Investigator must ensure that all site personnel involved in the trial are present during the SIV and will dedicate time for training. The SIV must be completed before the site can begin screening trial subjects.

To check compliance and verify the completeness, consistency and accuracy of the data being entered in the eCRFs and other protocol-related documents, monitoring visits will be performed to review source data versus the eCRFs. The assigned monitor will visit the Investigator and trial site at periodic intervals and maintain periodic communication. The Investigator agrees to allow the Monitor(s) and other authorized Sponsor personnel access to trial facilities and all source documents, as and when needed.

The Sponsor's monitoring standards require full verification that informed consent has been provided, verification of adherence to the inclusion/exclusion criteria, documentation and proper reporting of SAEs, and the recording of the trial endpoints. Additional checks will be performed according to the trial monitoring guidelines. The Monitor(s) will maintain 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 site staff.

While on site, the Monitor(s) will review the following documents, and the Investigator must ensure these documents are available for review by the Monitor:

- Source documents, in order to directly compare entries in the eCRF with the source documents
- Consenting procedures and documents
- Investigator Site File [ICH E6 (R2)]
- Other documentation verifying the activities conducted for the trial

The Monitor will ask for clarification and/or correction of any noted inconsistencies (procedures for correcting eCRFs are described in the eCRF completion guideline). The Investigator agrees to cooperate with the CRA(s) to ensure that any issues detected during these monitoring visits are resolved. As representatives of the Sponsor, monitors are responsible for notifying project management of any noted protocol deviations.

In compliance with ICH guidelines, the Investigator, or person designated by the Investigator, should document and explain any deviation from the approved protocol. The CRA should be informed of all deviations relating to trial inclusion/exclusion criteria, conduct of the trial, subject management, or subject assessment and ensure this is described and recorded in the relevant pages in the eCRF.

All laboratory data from biomarkers 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 with access to all trial documentation as described above, if requested. Further, the Investigator agrees to allow the Sponsor to assist inspectors and/or auditors in their duties, if requested.

A close-out visit will be performed for any initiated site when there are no more active subjects and after all follow-up issues have been resolved. If a site does not enroll any subjects, the close-out visit may be performed before trial database closure at the discretion of the Sponsor.

12 PUBLICATION AND DISSEMINATION POLICY

Data will be reported in a CTR in compliance with the requirements of the current version of ICH E3: "Structure and Content of Clinical Study Reports" [ICH E3, 1995].

The CTR will be issued within 12 months after the primary analysis the end of the trial and distributed as required by local regulations.

The addendum to the CTR 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 the EU Clinical Trials Register (www.clinicaltrialsregister.eu). A summary of the results, whether positive, negative, or inconclusive, will be published on ClinicalTrials.gov and the EU Clinical Trials Database.

The trial results may be published and presented to the public and/or used for educational purposes. The Sponsor shall have the sole and exclusive right to the first publication of the results of the trial, which is intended to be a multi-center publication of the trial results, collected from all Investigators and institutions participating in the trial. 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. Electronic 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 investigational product 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. Once the multi-center publication has been published (or if no such publication has occurred) within the time period established in the Clinical Trial Agreement (the "Agreement") since the completion or earlier termination of the trial at all participating sites (including the final database lock), the Investigator may publish the results of this trial obtained by him/her or otherwise present them at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor within the time period established in the Agreement for the Sponsor to provide comments and approval before the relevant publication submission deadline. If during this initial review period the Sponsor so requests, the Investigator shall delay the publication or presentation for an additional time period which allows the Sponsor to protect proprietary information.

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 detailed further in a separate document (the "Agreement"). In the event of any inconsistency between the Agreement and the protocol, the protocol shall prevail for

scientific matters and subject care issues, while the Agreement shall prevail for all other matters, including the publication and dissemination policy.

13 INSURANCE

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

Per local regulations, details about the insurance are described in the subject information sheet and ICF. A copy of the insurance certificate is filed in the ISF and the subject can request a copy.

14 FUNDING AND SUPPORT

A contract/agreement will be made (as appropriate) with the Investigator and/or institution.

The Sponsor will pay the trial sites through the CRO as per the site-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, subject hotel stay (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 payment to sites and reimbursement payments for subjects are also given in the Subject Information Letter and ICF.

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16 APPENDICES

16.1 Appendix: Scales and Assessments

16.1.1 EuroQol-5 Dimension – 5 Level Survey (EQ-5D-5L)

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

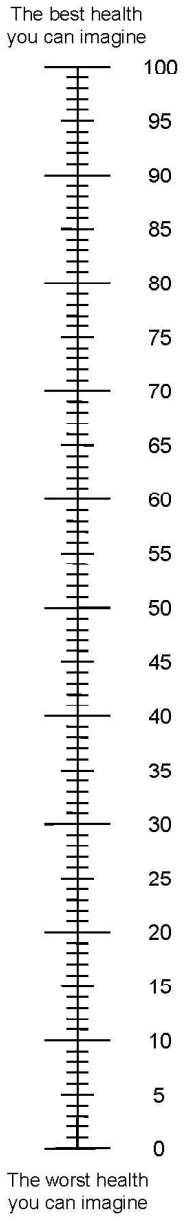
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Please mark an X on the scale to indicate how your health is TODAY.
- Now, write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



16.1.2 Functional Assessment of Cancer therapy – Bone Marrow Transplantation (FACT-BMT)

FACT-BMT (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

FACT-BMT (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-BMT (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
BMT1	I am concerned about keeping my job (include work at home)	0	1	2	3	4
BMT2	I feel distant from other people	0	1	2	3	4
BMT3	I worry that the transplant will not work	0	1	2	3	4
BMT4	The side effects of treatment are worse than I had imagined	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
C7	I like the appearance of my body	0	1	2	3	4
BMT5	I am able to get around by myself	0	1	2	3	4
BMT6	I get tired easily	0	1	2	3	4
BL4	I am interested in sex	0	1	2	3	4
BMT7	I have concerns about my ability to have children	0	1	2	3	4
BMT8	I have confidence in my nurse(s)	0	1	2	3	4
BMT9	I regret having the bone marrow transplant	0	1	2	3	4
BMT10	I can remember things	0	1	2	3	4
B1	I am able to concentrate	0	1	2	3	4
BMT11	I have frequent colds/infections	0	1	2	3	4
BMT12	My eyesight is blurry	0	1	2	3	4
BMT13	I am bothered by a change in the way food tastes	0	1	2	3	4
BMT14	I have tremors	0	1	2	3	4
B1	I have been short of breath	0	1	2	3	4
BMT15	I am bothered by skin problems	0	1	2	3	4
BMT16	I have trouble with my bowels	0	1	2	3	4
BMT17	My illness is a personal hardship for my close family members	0	1	2	3	4
BMT18	The cost of my treatment is a burden on me or my family	0	1	2	3	4

16.1.3 Patient Global Assessment of Severity

Please mark an X in the box that best describes the **severity** of your Graft Versus Host Disease (GVHD) symptoms overall **right now**.

Right now, my GVHD symptoms overall are:

- ☐ 0 Absent
- ☐ 1 Mild
- ☐ 2 Moderate
- ☐ 3 Severe
- ☐ 4 Extremely severe

16.1.4 Patient Global Assessment of Change

Patient Global Assessment of Change

Please mark an X in the box that best describes the **change** in your Graft Versus Host Disease (GVHD) symptoms overall **since you started taking the trial medication**.

☐ -2 A lot worse

☐ -1 A little bit worse

☐ 0 No change

☐ +1 A little bit improved

☐ +2 A lot improved

16.1.5 Physician Global Assessment of Disease

Please mark an X in the box that best describes your current overall severity of the patient's Graft Versus Host Disease (GVHD)

My overall assessment of my patient's disease severity is:

☐ 0 No sign of disease

☐ 1 Mild

☐ 2 Moderate

☐ 3 Severe

☐ 4 Extremely severe

16.2 Appendix: Definitions of Terms

The following definitions (derived from Supplementary Appendix in Zeiser, 2020a) will apply in this trial:

Term	Definition
Graft failure	Primary graft failure: Absolute neutrophil count <0.5 × 10^9/L by Day 28 Hemoglobin <80 g/L and platelets <20 × 10^9/L Reduced intensity conditioning: Confirmation of donor cell origin is required Cord blood transplant: Up to Day +42 Secondary graft failure: Absolute neutrophil count <0.5 × 10^9/L after initial engraftment not related to relapse, infection, or drug toxicity Reduced intensity conditioning: Loss of donor hematopoiesis to <5%
Failure-free survival	The time from the date of randomization/Day 0 to the date of hematologic malignancy relapse/progression, non-relapse mortality, or addition of new systemic acute graft versus host disease (aGVHD) treatment
Non-relapse mortality	The time from date of randomization/Day 0 to date of death not preceded by hematologic malignancy relapse/progression
Overall survival	The time from the date of randomization/Day 0 to the date of death due to any cause
Malignancy relapse/progression	The time from date of randomization/Day 0 to hematologic malignancy relapse/progression
Complete response	A score of 0 for the aGVHD grading in all evaluable organs that indicates complete resolution of all signs and symptoms of aGVHD in all evaluable organs without administration of additional systemic therapies for any earlier progression, mixed response or non-response of aGVHD
Complete lower gastrointestinal (GI)-aGVHD response	The resolution of all lower GI-aGVHD signs and symptoms GVHD without administration of additional systemic therapies for any earlier progression, mixed response, or non-response of lower GI-aGVHD
Partial response	An improvement of one Stage in one or more organs involved with aGVHD signs or symptoms without progression in other organs or sites without administration of additional systemic therapies for an earlier progression, mixed response, or non-response of aGVHD
Partial lower GI-aGVHD response	An improvement of one Stage in the signs and symptoms of lower GI-aGVHD without administration of additional systemic therapies for any earlier progression, mixed response, or non-response of lower GI-aGVHD
Lack of response	Defined as stable disease, mixed response, or progression
Stable disease	An absence of improvement in all organs involved by aGVHD, without worsening in all involved organs or development of signs or symptoms of aGVHD in a new organ

Stable disease (lower GI-aGVHD)	An absence of improvement or absence of worsening in lower GI-aGVHD
Mixed response	An improvement of at least one Stage in the severity of aGVHD in one organ accompanied by progression in another organ or development of signs or symptoms of aGVHD in a new organ
Progression	Worsening in one or more organs by one or more stages without improvement in any other involved organ
Lower GI-aGVHD progression	Worsening in lower GI-aGVHD as per the MAGIC lower GI-score compared with baseline
Lower GI-aGVHD flare	Any increase in signs or symptoms of lower GI-aGVHD that is sustained for >24 hours after apraglutide treatment completion following a CR or PR in the lower GI and requires re-escalation of immunosuppressive therapy (e.g., corticosteroid, calcineurin inhibitors and/or ruxolitinib dosing). While all aGVHD flares will be captured during the trial no matter the treatment, only flares that fulfil either one the following criteria will be considered a failure of treatment: 1. Addition of new systemic therapy for lower GI-aGVHD due to inability to taper corticosteroids below methylprednisolone (MP) 0.5 mg/kg/day (or equivalent <0.6 mg/kg/day of prednisone) for a minimum 7 days or 2. Addition of new systemic therapy for lower GI-aGVHD due to re-escalation of corticosteroids to MP >2 mg/kg/day (or equivalent >2.5 mg/kg/day of prednisone)
Best overall response	The best response recorded from the start of the treatment up to Day 91 or the start of additional systemic therapy for GI-aGVHD
Best overall lower GI-aGVHD response	The best response of lower GI-aGVHD response recorded from the start of the treatment up to Day 91 or the start of additional systemic therapy for GI-aGVHD

16.3 Appendix: Mount Sinai Acute Graft Versus Host Disease International Consortium (MAGIC) Grading Scale [Harris, 2016Harris, 2016]

Target Organ Staging

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (Stool Output/Day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	<500 mL/day or <3/episodes/day
1	Maculopapular rash <25% BSA	2–3 mg/dL	Persistent nausea, vomiting or anorexia	500–999 mL/day or 3–4/episodes/day
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dL		1000–1500 mL/day or 5–7/episodes/day
3	Maculopapular rash >50% BSA	6.1–15 mg/dL		>1500 mL/day or >7/episodes/day
4	Generalized erythroderma (>50% BSA) plus bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

BSA=body surface area; GI=gastrointestinal tract; GVHD=graft versus host disease

Overall Clinical Grade

Based on the most severe target organ involvement

Grade	Description
0	No Stage 1–4 of any organ
I	Stage 1–2 skin without liver, upper GI or lower GI involvement
II	Stage 3 rash and/or Stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI
III	Stage 2–3 liver and/or Stage 2–3 lower GI, with Stage 0–3 skin and/or Stage 0–1 upper GI
IV	Stage 4 skin, liver, or lower GI involvement, with Stage 0–1 upper GI

GI=gastrointestinal tract

16.4 Appendix: Summary of Changes to Previous Protocol Versions

Version No.	Version date	Changes to previous Protocol (amendments and indication of if substantial or not)
1.0		Not applicable, first version
1.2	14 Oct 2021	• Additional secondary objectives added to estimate duration of response from Day 56 and Day 29 • Clarification of inclusion criteria regarding how patients respond to corticosteroid and their stage of GI-aGVHD • The patient may now continue to take Apraglutide if there are no polyps detected at Week 13 and the investigator deems that the subject is benefitting from it • New Figure 1-2 added to show simulations of plasma Apraglutide and plasma Citrulline • New Figure 1-3 added to show low and high doses by body weight bands and respective estimated mean exposure • Dosing period updated from between 8 to 13 weeks to week 8 or week 13 • Definition of GI-aGVHD Flare included along with information regarding what should be done if a flare occurs • Greater clarification of how the injection will be performed • Greater clarification on dose reduction, modification and stopping rules of Apraglutide • Clarification of trial stopping criteria with three safety monitoring events identified: treatment-related deaths, grade 3–4 treatment-related organ toxicities and confirmed cases of Hy's law • Improved predictions made of the overall response rate to help determine sample size • Key secondary efficacy endpoint c will now be summarized using Kaplan-Meier analysis • Definitions of complete response, complete GIaGVHD response and partial response updated • Definitions of lack of response, no response, mixed response, and progression added to the definition

		section in Appendix 3
2.0	29 Oct 2021	• Minor administrative and grammatical changes throughout the protocol • Updated abbreviations list with minor wording changes • Addition of exclusion criteria to now exclude patients that have used enteral glutamine or growth factors such as GLP-2 within the previous 6 months to match existing concomitant medication restrictions • Updated planned timeline for first subject, first visit to March 2022 • Updated to show that telephone calls will not be made in weeks when a dosing visit is scheduled • Updated procedure list for the baseline visit to change the order procedures that take place • Updated procedure list for Visits 3–14 to change the order that procedures take place and to remove some procedures from visits e.g., removal of unnecessary extra nutrition status on Visit 9 and urine pregnancy test added to Visit 6 • Minimum data requirements to report an SAE changed to make reporting more simple • Updated reference list to include all papers cited in Version • Change to the pharmacokinetic information involving the removal of sampling at Day 90 • Adverse events collected throughout the study
3.0	25 Mar 2022	• Minor administrative and grammatical changes throughout the protocol • Updated abbreviations list and definitions throughout • Clarified the focus on lower GI-aGVHD • Updated the Background and Rationale of the Trial Synopsis, removing unessential information • Trial Objectives updated. Substantial changes made to Primary Objectives, Secondary Objectives and Exploratory Objectives • Trial Endpoints updated. Substantial changes made to overall endpoints; Safety Endpoints, Secondary Endpoints and Exploratory Endpoints • Trial design updated. Removal of repeat-dose

		definition of trial. • Extended RUX start to 0–5 days prior to first dose. • Removal of the following sentence: In case of transitory lack of tolerance, it is at the discretion of the treating physician to temporarily lower the dose • Inclusion/exclusion criteria updated • Inclusion/exclusion criteria clarified to refer to not only randomized but enrolled subjects as well • Measurements and procedures schedule updated. Deleted questionnaires collection at Visits 3 and 5. Added ECG and physical examination at Visit 2. Deleted therapeutic drug monitoring for administered immunosuppressants during treatment with apraglutide (collected as part of the concomitant medications • Statistical considerations updated within the Trial Synopsis • Trial duration clarified to up to 116 weeks • In Germany: oral methods of hormonal contraception are to be combined with another accepted method of contraception; only subjects 18 years old and above will be enrolled; only colonoscopies (not colonographies) can be performed • Figure 1-1 has been updated to incorporate a screening period beginning at Day -84 and incudes in the footnotes that dosing is not permitted at and after Day 182 • Table 1 updated style and formatting. Footnotes reflect changes made to the table • Section 1.4 updated to describe safety of the Phase II trial and expand upon safety of Phase I trial • Section 1.5 updated in keeping with the updates to the Trial Objectives and Endpoints seen in the Trial Synopsis • Section 1.9 updated to emphasize that the REACH 2 data was used for soft-powering finding a reasonable sample size. It was also added that the Sponsor will try to establish a better matched control • Section 2.1 updated to clarify that 3 dose groups will be assessed (not 5) and to emphasize that the Sponsor will try to establish a better matched control

		in regards to the study population and endpoints • Section 2.2 Overall design amended to read that dosing is not permitted at and after Day 182. Wording to this affect amended throughout the protocol as applicable • Trial Days updated to corresponding Weeks or Visits when applicable • The screening period cannot be extended beyond 12 weeks as aGVHD may have transitioned into cGVHD, in which case the subject will be classified as a screen failure • Historical data for colonographies/colonoscopies may be used where the colonoscopy/colonography was performed within 6 months before the screening visit • Historical data for biopsy may be used during screening for GI involvement confirmation, if obtained within 60 days prior to screening visit and after confirmed or suspected clinical diagnosis of GI-aGVHD. • For when treatment discontinuation may occur, the EOT visit will be performed 4 weeks after the last dose • Section 2.2.6 updated to outline the data that will be collected for subjects that are screen failures • Table 4 updated style and formatting, and several changes to dose administration under different circumstances • Clarified that individual apraglutide stopping events will not trigger iSRC meeting, but only as a part of Toxicity guidance rule calculation • Clarified that any investigational drug apart from apraglutide is prohibited during the trial • After reconstitution, the IMP must be injected within a maximum of 2 hours from completing reconstitution in order to ensure sterility is maintained • The FACT-BMT will only be completed by subjects aged ≥18 years old • Clarified that blood transfusions should be recorded as a concomitant procedure in the eCRF • Where apraglutide was not administered during the previous week, e.g., for safety reason, the pre-dose

		PK sample at the current visit can be skipped • Clarified that dosing visits may also occur in Weeks: 1416, 1820, 22-25 • Ideal dates of Visits 9, 10, 11, 12, 13, 14 were clarified • Serious adverse events related to trial medication (in the Investigator's opinion) to be reported after the EOT visit • If an AE or MH event worsens in severity or frequency during the trial, then a new AE is to be registered with the date of the worsening as the start date • Hypersensitivity and anaphylaxis (narrow SMQs) will be collected as AESIs • Clarified that subjects may only reduce their dose by one step during the trial • Toxicity Events identified for monitoring were clarified • Table 13 replaced with text describing the probability of higher effectiveness with apraglutide relative to the best available therapy • For every subject, visual inspection of the actual concentrations will be compared to a simulated PK profile based on a PK model developed on healthy volunteers and SBS patients' data from Phase I and II clinical studies. The simulated PK profile will be constructed based on the dose received and the subject's body weight. This will be performed online for every subject, and this will be used to check if the subject is correctly exposed to apraglutide • Section 7.7.1 updated in keeping with the updates to the Endpoints in the Trial Synopsis • Section 7.7.2 updated in keeping with the updates to the Endpoints in the Trial Synopsis • Section 7.7.3 incorporated into Section 7.7.2 • Section 7.7.4 renumbered to: 7.7.3, and all information replaced with reference to the FAS and SAP • Updated reference list to remove references no longer applicable to the updated Version 3.0 content • Several terms and definitions added to Appendix 3:

		Stable disease (lower GI-aGVHD); Lower GI-aGVHD progression; Best overall lower GI-aGVHD response • Appendix 7 was added to present the flowcharts on adjustment of apraglutide and RUX doses
4.0	26 Sept 2022	Summary of changes are provided in the separate Protocol Amendment document.
5.0	27 Mar 2023	Summary of changes are provided in the separate Protocol Amendment document.